

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 February 2002 (07.02.2002)

PCT

(10) International Publication Number
WO 02/10382 A2

(51) International Patent Classification⁷: C12N 15/12, 15/11, 9/00, C07K 14/47, C12Q 1/68, G01N 33/577, A61K 31/713

(21) International Application Number: PCT/EP01/08309

(22) International Filing Date: 18 July 2001 (18.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/221,513 28 July 2000 (28.07.2000) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 02/10382 A2

(54) Title: TRP8, TRP9 AND TRP10, NOVEL MARKERS FOR CANCER

(57) Abstract: The present invention relates to gene expression in normal cells and cells of malignant tumors and particularly to novel markers associated with cancer, Trp8, Trp9 and Trp10, and the genes encoding Trp8, Trp9 and Trp10. Also provided are vectors, host cells, antibodies, and recombinant methods for producing these human proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating a tumor.

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Trp8, Trp9 and Trp10, novel markers for cancer**FIELD OF THE INVENTION**

The present invention relates to gene expression in normal cells and cells of malignant tumors and particularly to novel markers associated with cancer, Trp8, Trp9 and Trp10, and the genes encoding Trp8, Trp9 and Trp10

BACKGROUND OF THE TECHNOLOGY

Prostate cancer is one of the most common diseases of older men world wide. Diagnosis and monitoring of prostate cancer is difficult because of the heterogeneity of the disease. For diagnosis different grades of malignancy can be distinguished according to the Gleason-Score Diagnosis. For this diagnosis a prostate tissue sample is taken from the patient by biopsy and the morphology of the tissue is investigated. However, this approach only yields subjective results depending on the experience of the pathologist. For confirmation of these results and for obtaining an early diagnosis an additional diagnostic method can be applied which is based on the detection of a prostate specific antigen (PSA). PSA is assayed in serum samples, blood samples etc. using an anti-PSA-antibody. However, since in principle PSA is also expressed in normal prostate tissue there is a requirement for the definition of a threshold value (about 4 ng/ml PSA) in order to be able to distinguish between normal and malign prostate tissue. Unfortunately, this diagnostic method is quite insensitive and often yields false-positive results. Moreover, by using this diagnostic method any conclusions as regards the grade of malignancy, the progression of the tumor and its potential for metastasizing cannot be drawn. Thus, the use of molecular markers would be helpful to distinguish benign from malign tissue and for grading and staging prostate carcinoma, particularly for patients with metastasizing prostate cancer having a very bad prognosis.

The above discussed limitations and failings of the prior art to provide meaningful specific markers which correlate with the presence of prostate tumors, in particular metastasizing tumors, has created a need for markers which can be used diagnostically, prognostically and therapeutically over the course of this disease. The present invention fulfils such a need by the provision of Trp8, Trp9 and Trp10 and the genes encoding Trp8, Trp9 and Trp10: The genes encoding Trp8 and Trp10 are expressed in prostate carcinoma and prostatic metastasis, but

not in normal prostate, benign hyperplasia (BHP) and intraepithelial prostatic neoplasia (PIN). Furthermore, expression of Trp10 transcripts is detectable in carcinoma but not in healthy tissue of the lung, the prostate, the placenta and in melanoma.

SUMMARY OF THE INVENTION

The present invention is based on the isolation of genes encoding novel markers associated with cancer, Trp8, Trp9 and Trp10. The new calcium channel proteins Trp8, Trp9 and Trp10 are members of the trp (transient receptor potential) - family, isolated from human placenta (Trp8a and Trp8b) and humane prostate (Trp9, Trp10a and Trp10b). Trp proteins belong to a steadily growing family of Ca^{2+} selective and non selective ion channels. In the recent years seven Trp proteins (trp1 - trp7) have been identified and suggested to be involved in cation entry, receptor operated calcium entry and pheromone sensory signaling. Structurally related to the trp proteins are the vanilloid receptor (VR1) and the vanilloid like receptor (VRL-1) both involved in nociception triggered by heat. Furthermore, two calcium permeable channels were identified in rat small intestine (CaT1) and rabbit kidney (ECaC). These distantly related channels are suggested to be involved in the uptake of calcium ions from the lumen of the small intestine (CaT1) or in the reuptake of calcium ions in the distal tubule of the kidney (ECaC). Common features of the Trp and related channels are a proposed structure comprising six transmembrane domains including several conserved amino acid motifs. In the present invention the cloning and expression of a CaT1 like calcium channel (Trp8) from human placenta as well as Trp9 and Trp10 (two variants, Trp10a and Trp10b) is described. Two polymorphic variants of the Trp8 cDNA were isolated from placenta (Trp8a and Trp8b). Transient expression of the Trp8b cDNA in HEK (human embryonic kidney) cells results in cytosolic calcium overload implicating that the Trp8 channel is constitutive open in the expression system. Trp8 induces highly calcium selective inward currents in HEK cells. The C-terminus of the Trp8 protein binds calmodulin in a calcium dependent manner. The Trp9 channel is expressed in trophoblasts and syncytiotrophoblasts of placenta and in pancreatic acinar cells. Furthermore, the Trp8 channel is expressed in prostatic carcinoma and prostatic metastases, but not in normal tissue of the prostate. No expression of Trp8 transcripts is detectable in benign prostatic hyperplasia (BPH) or prostatic intraepithelial neoplasia (PIN). Therefore, the Trp8 channel is exclusively expressed in malign prostatic tissues and serves as molecular marker for prostate cancer. From the experimental results it is also apparent that the

modulation of Trp8 and/or Trp10, e.g. the inhibition of expression or activity, is of therapeutic interest, e.g. for the prevention of tumor progression.

The present invention, thus, provides a Trp8, Trp9 and Trp10 protein, respectively, as well as nucleic acid molecule encoding the protein and, moreover, an antisense RNA, a ribozyme and an inhibitor, which allow to inhibit the expression or the activity of Trp8, Trp9 and/or Trp10.

In one embodiment, the present invention provides a diagnostic method for detecting a prostate cancer or endometrial cancer (cancer of the uterus) associated with Trp8 or Trp10 in a tissue of a subject, comprising contacting a sample containing Trp8 and/or Trp10 encoding mRNA with a reagent which detects Trp8 and/or Trp10 or the corresponding mRNA.

In a further embodiment, the present invention provides a diagnostic method for detecting a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense transcripts or Trp10a and/or Trp10b related antisense transcripts.

In another embodiment, the present invention provides a method of treating a prostate tumor, carcinoma of the lung, carcinoma of the placenta (chorion carcinoma) or melanoma associated with Trp8 and/or Trp10, comprising administering to a subject with such an disorder a therapeutically effect amount of a reagent which modulates, e.g. inhibits, expression of Trp8 and/or Trp10 or the activity of the protein, e.g. the above described compounds.

Finally, the present invention provides a method of gene therapy comprising introducing into cells of a subject an expression vector comprising a nucleotide sequence encoding the above mentioned antisense RNA or ribozyme, in operable linkage with a promoter.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: A, phylogenetic relationship of trp and related proteins. B, hydropathy plot of the Trp8 protein sequence according to Kyte and Doolittle. C, alignment of Trp8a/b to the epithelial calcium channels ECaC (from rabbit) and Vr1 (from rat). Putative transmembrane domains are underlined.

Figure 2: A, polymorphism of the Trp8 gene. The polymorphic variants Trp8a and Trp8b differ in five base pairs resulting in three amino acid exchanges in the derived protein sequences. Specific primers were derived from the Trp8 gene as indicated by arrows. B, the Trp8a and Trp8b genes are distinguishable by a single restriction site. Genomic fragments of the Trp8 gene can be amplified using specific primers (shown in A). The genomic fragment of the Trp8b gene contains an additional site of the restriction enzyme BSP1286I (B). C, the Trp8 gene is located on chromosome 7. D, genotyping of eleven human subjects. A 458 bp genomic fragment of the Trp8 gene was amplified using specific primers (shown in A) and restricted with BSP1286I. The resulting fragments were analyzed by PAGE electrophoresis.

Figure 3: The Trp8b protein is a calcium selective ion channel. A, representative trace of a pdiTrp8b transfected HEK 293 cell. Trp8b mediated currents are activated by voltage ramps (-100 mV - +100 mV) of 100 msec at -40 mV or +70 mV holding potential. 1, Trp8b currents in the presence at 2mM $[Ca^{2+}]_o$; 2, effect of solution switch alone 3, switch to nominal zero calcium solution. B, Trp8b currents in the presence of zero divalent cations. C, current voltage relationship of the currents shown in A. Inset, leak subtracted current. D, current voltage relationship of the current shown in B. E, statistics of representative experiments. Black: Trp8 transfected cells, gray: control cells. Columns from left to right: Trp8 currents at -40 mV (n=12) and +70 mV holding potential (n=12). Trp8 currents in standard bath solution including 120 mM NMDG without sodium (n=7) and with nominal zero calcium ions (n=8) or in the presence of 1mM EGTA with zero divalent cations (n=6). F, representative changes in $[Ca^{2+}]_i$ in Trp8b transfected HEK cells (gray) and controls (black) in the presence or absence of 1mM $[Ca^{2+}]_o$. Inset, relative increase of cytosolic calcium concentration of Trp8b transfected HEK cells, before and after readdition of 1 mM $[Ca^{2+}]_o$ in comparison to control cells.

Figure 4: The C-terminal region of the Trp8 protein binds calmodulin. A, N- and C-terminal fragments of the Trp8 protein used for calmodulin binding studies. B, the Trp8 protein and a truncated Trp8 protein which was in vitro translated after MunI cut of the cDNA, which lacks the C-terminal 32 amino acid residues, were in vitro translated in the presence of ^{35}S -methionine and incubated with calmodulin coupled agarose beads in the presence of 1 mM Ca^{2+} or 2 mM EGTA. C, calmodulin binding to N- and C-terminal fragments of the Trp8 protein in the presence of Ca^{2+} (1 mM) or EGTA (2 mM)

Figure 5: Expression pattern of the Trp8 cDNA. A, Northern blots (left panels, Clontech, Palo Alto) were hybridized using a 348 bp NcoI/BamHT fragment of the Trp9 cDNA. The probe hybridizes to mRNA species isolated from the commercial blot, but not to mRNA species isolated from benign prostate hyperplasia (right panel, mRNA isolated from 20 human subjects with benign prostate hyperplasia). B,C, in situ hybridization with biotinylated Trp8 specific oligonucleotides on slides of human tissues. Left column antisense probes, right column sense probes. D, antinsense probes.

Figure 6: Differential expression of Trp8 cDNA in human prostate. A - F, in situ hybridization with prostatic tissues. A, normal prostate, B, primary carcinoma, C, benign hyperplasia, D, rezidive carcinoma, E, prostatic intraepithelial neoplasia, F, lymphnode metastasis of the prostata.

Figure 7: Trp8a cDNA sequence and derived amino acid sequence

Figure 8: A, Trp8b cDNA sequence and derived amino acid sequence

B, cDNA sequence of splice variant 1 (12B1)

C, cDNA sequence of splice variant 2 (17-3)

D, cDNA sequence of splice variant 3 (23A3)

E, cDNA sequence of splice variant 4 (23C3)

Figure 9: A, Trp9 cDNA sequence and derived amino acid sequence B, cDNA sequence of splice variant 15 and derived amino acid sequence. .

Figure 10: A, cDNA sequence of Trp10a and derived amino acid sequence, B, cDNA fragment of Trp10a and derived amino acid sequence.

Figure 11: cDNA sequence of Trp10b and derived amino acid sequence.

Figure 12: Expression of Trp8 mRNA in human endometrial cancer or cancer of the uterus. A - D, in situ hybridization with slides of endometrial cancer hybridized with Trp8 antisense (left column) or sense probes as controls (right column). E - F, Trp8 antisense probes hybridized to slides of normal endometrium. It can be clearly seen no hybridization occurs with normal endometrial tissue.

Figure 13: Expression of human Trp9 and Trp10 genes

Northern blots were hybridized using Trp9 (upper panel) or Trp10 (lower panel) specific probes. Expression of the Trp9 cDNA is detectable in many tissues including human prostate and colon as well as in benign prostatic hyperplasia. Expression of Trp10 cDNA is detectable in human prostate of a commercial northern blot (Clontech, right side). This Northern blot contains prostatic tissue collected from 15 human subjects in the range of 14 - 60 years of age. No expression of Trp10 cDNA was detectable in benign prostatic hyperplasia (left side).

Figure 14: Expression of Trp10 transcripts and Trp10-antisense transcripts in human prostate cancer and metastasis of a melanoma. In situ hybridizations of slides hybridized with Trp10-antisense (A-E, K-N) and Trp10 related sense probes (F-J, P-R). It can clearly be seen that both probes detect the same cancer cells indicating that these cancer cells express Trp10 transcripts as well as Trp10-antisense transcripts. S, no Trp10 expression is detectable in benign hyperplasia of the prostate (BPH). O and T, show expression of Trp10 transcripts (O) and Trp10-antisense transcripts (T) in a metastasis of a melanoma in human lung. Melanoma cancer cells express both Trp10 transcripts and Trp10-antisense transcripts.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b or a protein exhibiting biological properties of Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being selected from the group consisting of

- (a) a nucleic acid molecule encoding a protein that comprises the amino acid sequence depicted in Figure 7, 8A, 9,10 or 11;
- (b) a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9,10, or 11;
- (c) a nucleic acid molecule included in DSMZ Deposit no. DSM 13579 (deposit date: 28 June 2000), DSM 13580 (deposit date: 28 June 2000), DSM 13584 (deposit date: 5 July 2000), DSM 13581 (deposit date: 28 June 2000) or DSM(deposit date:....);
- (d) a nucleic acid molecule with hybridizes to a nucleic acid molecule specified in (a) to (c)

- (e) a nucleic acid molecule the nucleic acid sequence of which deviates from the nucleic sequences specified in (a) to (d) due to the degeneration of the genetic code; and
- (f) a nucleic acid molecule, which represents a fragment, derivative or allelic variation of a nucleic acid sequence specified in (a) to (e).

As used herein, a protein exhibiting biological properties of Trp8a, Trp8b, Trp9, Trp10a or Trp10b is understood to be a protein having at least one of the activities as illustrated in the Examples, below.

As used herein, the term „isolated nucleic acid molecule,, includes nucleic acid molecules substantially free of other nucleic acids, proteins, lipids, carbohydrates or other materials with which it is naturally associated.

In a first embodiment, the invention provides an isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b comprising the amino acid sequence depicted in Figure 7, 8A, 9, 10 or 11. The present invention also provides a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9, 10 or 11.

The present invention provides not only the generated nucleotide sequence identified in Figure 7, 8A, 9, 10 or 11, respectively and the predicted translated amino acid sequence, respectively, but also plasmid DNA containing a Trp8a cDNA deposited with the DSMZ, under DSM 13579, a Trp8b cDNA deposited with the DSMZ, under DSM 13580, a Trp9 cDNA deposited with the DSMZ, under DSM 13584, a Trp10a cDNA deposited with the DSMZ, under DSM 13581, and a Trp10b cDNA deposited with the DSMZ, under DSM..., respectively. The nucleotide sequence of each deposited Trp-clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by each deposited clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited Trp-encoding DNA, collecting the protein, and determining its sequence.

The nucleic acid molecules of the invention can be both DNA and RNA molecules. Suitable DNA molecules are, for example, genomic or cDNA molecules. It is understood that all

nucleic acid molecules encoding all or a portion of Trp8a, Trp8b, Trp9, Trp10a or Trp10b are also included, as long as they encode a polypeptide with biological activity. The nucleic acid molecules of the invention can be isolated from natural sources or can be synthesized according to known methods.

The present invention also provides nucleic acid molecules which hybridize to the above nucleic acid molecules. As used herein, the term „hybridize,, has the meaning of hybridization under conventional hybridization conditions, preferably under stringent conditions as described, for example, in Sambrook et al., Molecular Cloning, A Laboratory Manual 2nd edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Also contemplated are nucleic acid molecules that hybridize to the Trp nucleic acid molecules at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency), salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 9.2M NaH₂PO₄; 0.02M EDTA, pH7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA, following by washes at 50°C with 1 X SSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC). Variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Nucleic acid molecules that hybridize to the molecules of the invention can be isolated, e.g., from genomic or cDNA libraries that were produced from human cell lines or tissues. In order to identify and isolate such nucleic acid molecules the molecules of the invention or parts of these molecules or the reverse complements of these molecules can be used, for example by means of hybridization according to conventional methods (see, e.g., Sambrook et al., supra). As a hybridization probe nucleic acid molecules can be used, for example, that have exactly or basically the nucleotide sequence depicted in Figure 7, 8A, 9, 10 or 11, respectively, or parts of these sequences. The fragments used as hybridization probe can be synthetic

fragments that were produced by means of conventional synthetic methods and the sequence of which basically corresponds to the sequence of a nucleic acid molecule of the invention.

The nucleic acid molecules of the present invention also include molecules with sequences that are degenerate as a result of the genetic code.

In a further embodiment, the present invention provides nucleic acid molecules which comprise fragments, derivatives and allelic variants of the nucleic acid molecules described above encoding a protein of the invention. „Fragments,, are understood to be parts of the nucleic acid molecules that are long enough to encode one of the described proteins. These fragments comprise nucleic acid molecules specifically hybridizing to transcripts of the nucleic acid molecules of the invention. These nucleic acid molecules can be used, for example, as probes or primers in the diagnostic assay and/or kit described below and, preferably, are oligonucleotides having a length of at least 10, in particular of at least 15 and particularly preferred of at least 50 nucleotides. The nucleic acid molecules and oligonucleotides of the invention can also be used, for example, as primers for a PCR reaction. Examples of particular useful probes (primers) are shown in Tables 1 and 2.

Table 1

Trp8 probes used for in situ hybridization:

Probes (antisense)

- 1.) 5' TCCGCTGCCGGTTGAGATCTTGCC 3'
- 2.) 5' CTTGCTCCATAGGCAGAGAATTAG 3'
- 3.) 5' ATCCTCAGAGCCCCGGGTGTGGAA3'

Controls (sense)

- 1.) 5' GGCAAGATCTCAACCGGCAGCGGA 3'
- 2.) 5' CTAATTCTCTGCCTATGGAGCAAG 3'
- 3.) 5' TTCCACACCCGGGGCTCTGAGGAT 3'

Tabelle 2

Trp10 probes used for the in situ hybridizations shown in Figure 14:

Probes (antisense)

- 1.) 5' GCTTCCACCCCAAGCTTCACAGGAATAGA 3' (Figure 14 A, 14B)
- 2.) 5' GGCGATGAAATGCTGGTCTGTGGC 3' (Figure 14C, 14D, 14N, 14S, 14O)
- 3.) 5' ATCTTCCAGTTCTTGGTGTCTCGG 3' (Figure 14E, 14K)
- 4.) 5' GCTGCAGTACTCCTGCACCAGGAA 3' (Figure 14L, 14M)

Probes (sense)

- 1.) 5' TCTATTCCTGTGAAGCTTGGGGTGGGAAGC 3' (Figure 14F, 14G)
- 2.) 5' GCCACAGACCAGCATTTCATCGCC 3' (Figure 14H, 14I, 14T)
- 3.) 5' CCGAGACACCAAGAACTGGAAGAT 3' (Figure 14J, 14P)
- 4.) 5' TTCCTGGTGCAGGAGTACTGCAGC 3' (Figure 14Q, 14R)

The term „derivative,, in this context means that the sequences of these molecules differ from the sequences of the nucleic acid molecules described above at one or several positions but have a high level of homology to these sequences. Homology hereby means a sequence identity of at least 40%, in particular an identity of at least 60%, preferably of more than 80% and particularly preferred of more than 90%. These proteins encoded by the nucleic acid molecules have a sequence identity to the amino acid sequence depicted in Figure 7, 8A, 9, 10 and 11, respectively, of at least 80%, preferably of 85% and particularly preferred of more than 90%, 97% and 99%. The deviations to the above-described nucleic acid molecules may have been produced by deletion, substitution, insertion or recombination. The definition of the derivatives also includes splice variants, e.g. the splice variants shown in Figures 8B to 8E and 9B.

The nucleic acid molecules that are homologous to the above-described molecules and that represent derivatives of these molecules usually are variations of these molecules that represent modifications having the same biological function. They can be naturally occurring variations, for example sequences from other organisms, or mutations that can either occur naturally or that have been introduced by specific mutagenesis. Furthermore the variations can be synthetically produced sequences. The allelic variants can be either naturally occurring variants or synthetically produced variants or variants produced by recombinant DNA processes.

Generally, by means of conventional molecular biological processes it is possible (see, e.g., Sambrook et al., supra) to introduce different mutations into the nucleic acid molecules of the invention. As a result Trp proteins or Trp related proteins with possibly modified biological properties are synthesized. One possibility is the production of deletion mutants in which nucleic acid molecules are produced by continuous deletions from the 5'- or 3'-terminal of the coding DNA sequence and that lead to the synthesis of proteins that are shortened accordingly. Another possibility is the introduction of single-point mutation at positions where a modification of the amino acid sequence influences, e.g., the ion channel properties or the regulations of the trp-ion channel. By this method muteins can be produced, for example, that possess a modified ion conducting pore, a modified K_m -value or that are no longer subject to the regulation mechanisms that normally exist in the cell, e.g. with regard to allosteric regulation or covalent modification. Such muteins might also be valuable as therapeutically useful antagonists of Trp8a, Trp8b, Trp9, Trp10a or Trp10b, respectively.

For the manipulation in prokaryotic cells by means of genetic engineering the nucleic acid molecules of the invention or parts of these molecules can be introduced into plasmids allowing a mutagenesis or a modification of a sequence by recombination of DNA sequences. By means of conventional methods (cf. Sambrook et al., supra) bases can be exchanged and natural or synthetic sequences can be added. In order to link the DNA fragments with each other adapters or linkers can be added to the fragments. Furthermore, manipulations can be performed that provide suitable cleavage sites or that remove superfluous DNA or cleavage sites. If insertions, deletions or substitutions are possible, in vitro mutagenesis, primer repair, restriction or ligation can be performed. As analysis method usually sequence analysis, restriction analysis and other biochemical or molecular biological methods are used.

The proteins encoded by the various variants of the nucleic acid molecules of the invention show certain common characteristics, such as ion channel activity, molecular weight, immunological reactivity or conformation or physical properties like the electrophoretical mobility, chromatographic behavior, sedimentation coefficients, solubility, spectroscopic properties, stability; pH optimum, temperature optimum.

The invention furthermore relates to vectors containing the nucleic acid molecules of the invention. Preferably, they are plasmids, cosmids, viruses, bacteriophages and other vectors

usually used in the field of genetic engineering. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in mammalian cells and baculovirus-derived vectors for expression in insect cells. Preferably, the nucleic acid molecule of the invention is operatively linked to the regulatory elements in the recombinant vector of the invention that guarantee the transcription and synthesis of an RNA in prokaryotic and/or eukaryotic cells that can be translated. The nucleotide sequence to be transcribed can be operably linked to a promoter like a T7, metallothionein I or polyhedrin promoter.

In a further embodiment, the present invention relates to recombinant host cells transiently or stable containing the nucleic acid molecules or vectors or the invention. A host cell is understood to be an organism that is capable to take up *in vitro* recombinant DNA and, if the case may be, to synthesize the proteins encoded by the nucleic acid molecules of the invention. Preferably, these cells are prokaryotic or eukaryotic cells, for example mammalian cells, bacterial cells, insect cells or yeast cells. The host cells of the invention are preferably characterized by the fact that the introduced nucleic acid molecule of the invention either is heterologous with regard to the transformed cell, i.e. that it does not naturally occur in these cells, or is localized at a place in the genome different from that of the corresponding naturally occurring sequence.

A further embodiment of the invention relates to isolated proteins exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being encoded by the nucleic acid molecules of the invention, as well as to methods for their production, whereby, e.g., a host cell of the invention is cultivated under conditions allowing the synthesis of the protein and the protein is subsequently isolated from the cultivated cells and/or the culture medium. Isolation and purification of the recombinantly produced proteins may be carried out by conventional means including preparative chromatography and affinity and immunological separations involving affinity with an anti-Trp8a-, anti-Trp8b-, anti-Trp9-, anti-Trp10a- or anti-Trp10b-antibody, respectively.

As used herein, the term „isolated protein,, includes proteins substantially free of other proteins, nucleic acids, lipids, carbohydrates or other materials with which it is naturally associated. Such proteins however not only comprise recombinantly produced proteins but include isolated naturally occurring proteins, synthetically produced proteins, or proteins

produced by a combination of these methods. Means for preparing such proteins are well understood in the art. The Trp proteins are preferably in a substantially purified form. A recombinantly produced version of a human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b protein, including the secreted protein, can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67; 31-40 (1988).

In a further preferred embodiment, the present invention relates to an antisense RNA sequence characterised that it is complementary to an mRNA transcribed from a nucleic acid molecule of the present invention or a part thereof and can selectively bind to said mRNA, said sequence being capable of inhibiting the synthesis of the protein encoded by said nucleic acid molecules, and a ribozyme characterised in that it is complementary to an mRNA transcribed from a nucleic acid molecule of the present invention or a part thereof and can selectively bind to and cleave said mRNA, thus inhibiting the synthesis of the proteins encoded by said nucleic acid molecules. Ribozymes which are composed of a single RNA chain are RNA enzymes, i.e. catalytic RNAs, which can intermolecularly cleave a target RNA, for example the mRNA transcribed from one of the Trp genes. It is now possible to construct ribozymes which are able to cleave the target RNA at a specific site by following the strategies described in the literature. (see, e.g., Tanner et al., in: *Antisense Research and Applications*, CRC Press Inc. (1993), 415-426). The two main requirements for such ribozymes are the catalytic domain and regions which are complementary to the target RNA and which allow them to bind to its substrate, which is a prerequisite for cleavage. Said complementary sequences, i.e., the antisense RNA or ribozyme, are useful for repression of Trp8a-, Trp8b, Trp9-, Trp10a- and Trp10b-expression, respectively, i.e. in the case of the treatment of a prostate cancer or endometrial cancer (carcinoma of the uterus). Preferably, the antisense RNA and ribozyme of the invention are complementary to the coding region. The person skilled in the art provided with the sequences of the nucleic acid molecules of the present invention will be in a position to produce and utilise the above described antisense RNAs or ribozymes. The region of the antisense RNA and ribozyme, respectively, which shows complementarity to the mRNA transcribed from the nucleic acid molecules of the present invention preferably has a length of at least 10, in particular of at least 15 and particularly preferred of at least 50 nucleotides.

In still a further embodiment, the present invention relates to inhibitors of Trp8a, Trp8b, Trp9, Trp10a and Trp10b, respectively, which fulfill a similar purpose as the antisense RNAs or

ribozymes mentioned above, i.e. reduction or elimination of biologically active Trp8a, Trp8b, Trp9, Trp10a or Trp10b molecules. Such inhibitors can be, for instance, structural analogues of the corresponding protein that act as antagonists. In addition, such inhibitors comprise molecules identified by the use of the recombinantly produced proteins, e.g. the recombinantly produced protein can be used to screen for and identify inhibitors, for example, by exploiting the capability of potential inhibitors to bind to the protein under appropriate conditions. The inhibitors can, for example, be identified by preparing a test mixture wherein the inhibitor candidate is incubated with Trp8a, Trp8b, Trp9, Trp10a or Trp10b, respectively, under appropriate conditions that allow Trp8a, Trp8b, Trp9, Trp10a or Trp10b to be in a native conformation. Such an in vitro test system can be established according to methods well known in the art. Inhibitors can be identified, for example, by first screening for either synthetic or naturally occurring molecules that bind to the recombinantly produced Trp protein and then, in a second step, by testing those selected molecules in cellular assays for inhibition of the Trp protein, as reflected by inhibition of at least one of the biological activities as described in the examples, below. Such screening for molecules that bind Trp8a, Trp8b, Trp9, Trp10a or Trp10b could easily be performed on a large scale, e.g. by screening candidate molecules from libraries of synthetic and/or natural molecules. Such an inhibitor is, e.g., a synthetic organic chemical, a natural fermentation product, a substance extracted from a microorganism, plant or animal, or a peptide. Additional examples of inhibitors are specific antibodies, preferably monoclonal antibodies. Moreover, the nucleic acid sequences of the invention and the encoded proteins can be used to identify further factors involved in tumor development and progression. In this context it should be emphasized that the modulation of the calcium channel of a member of the trp family can result in the stimulation of the immune response of T lymphocytes leading to proliferation of the T lymphocytes. The proteins of the invention can, e.g., be used to identify further (unrelated) proteins which are associated with the tumor using screening methods based on protein/protein interactions, e.g. the two-hybrid-system Fields, S. and Song, O. (1989) *Nature* (340): 245-246.

The present invention also provides a method for diagnosing a prostate carcinoma which comprises contacting a target sample suspected to contain the protein Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA with a reagent which reacts with Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA and detecting Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA.

It has been found that carcinoma cells of placenta (chorion carcinoma), lung and prostate express Trp10 transcripts as well as Trp10 antisense transcripts and transcripts being in part complementary to Trp10 antisense transcripts. Accordingly, the present invention also provides a method for diagnosing a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense RNA.

When the target is mRNA (or antisense RNA), the reagent is typically a nucleic acid probe or a primer for PCR. The person skilled in the art is in a position to design suitable nucleic acids probes based on the information as regards the nucleotide sequence of Trp8a, Trp8b, Trp10a or Trp10b as depicted in figure 7, 8a, 10 and 11, respectively, or tables 1 and 2, above. When the target is the protein, the reagent is typically an antibody probe. The term „antibody“, preferably, relates to antibodies which consist essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations. Monoclonal antibodies are made from an antigen containing fragments of the proteins of the invention by methods well known to those skilled in the art (see, e.g., Köhler et al., *Nature* 256 (1975), 495). As used herein, the term „antibody“ (Ab) or „monoclonal antibody“ (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')₂ fragments) which are capable of specifically binding to protein. Fab and f(ab')₂ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. (Wahl et al., *J. Nucl. Med.* 24: 316-325 (1983)). Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimerical, single chain, and humanized antibodies. The target cellular component, i.e. Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts, e.g., in biological fluids or tissues, may be detected directly in situ, e.g. by in situ hybridization (e.g., according to the examples, below) or it may be isolated from other cell components by common methods known to those skilled in the art before contacting with a probe. Detection methods include Northern blot analysis, RNase protection, in situ methods, e.g. in situ hybridization, in vitro amplification methods (PCR, LCR, QRNA replicase or RNA-transcription/amplification (TAS, 3SR), reverse dot blot disclosed in EP-B1 O 237 362)), immunoassays, Western blot and other detection assays that are known to those skilled in the art.

Products obtained by in vitro amplification can be detected according to established methods, e.g. by separating the products on agarose gels and by subsequent staining with ethidium bromide. Alternatively, the amplified products can be detected by using labeled primers for amplification or labeled dNTPs.

The probes can be detectable labeled, for example, with a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.

Expression of Trp8a, Trp8b, Trp10a and Trp10b, respectively, in tissues can be studied with classical immunohistological methods (Jalkanen et al., J. Cell. Biol. 101 (1985), 976-985; Jalkanen et al., J. Cell. Biol. 105 (1987), 3087-3096; Sobol et al. Clin. Immunopathol. 24 (1982), 139-144; Sobol et al., Cancer 65 (1985), 2005-2010). Other antibody based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{112}In), and technetium rhodamine, and biotin. In addition to assaying Trp8a, Trp8b, Trp 10a or Trp10b levels in a biological sample, the protein can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma. A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I , ^{112}In , ^{99}mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99}mTc . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In

vivo tumor imaging is described in S.W. Burchiel et al., „Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments“. (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B.A. Rhodes, eds., Masson Publishing Inc. (1982)).

The marker Trp8a and Trp8b is also useful for prognosis, for monitoring the progression of the tumor and the diagnostic evaluation of the degree of malignancy of a prostate tumor (grading and staging), e.g. by using in situ hybridization: In a primary carcinoma Trp8 is expressed in about 2 to 10% of carcinoma cells, in a rezidive carcinoma in about 10 to 60% of cells and in metastases in about 60 to 90% of cells.

The present invention also relates to a method for diagnosing endometrial cancer (cancer of the uterus) which comprises contacting a target sample suspected to contain the protein Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA with a reagent which reacts with Trp8a and/or Trp8b or the encoding mRNA and detecting Trp8a and/or Trp8b encoding mRNA. As regards particular embodiments of this method reference is made to the particular embodiments of the method of diagnosing a prostate cancer outlined above.

For evaluating whether the concentration of Trp8a, Trp8b, Trp10a or Trp10b or the concentration of Trp8a, Trp8b, Trp10a or Trp10b encoding mRNA is normal or increased, thus indicative for the presence of a malignant tumor, the measured concentration is compared with the concentration in a normal tissue, preferably by using the ratio of Trp8a:Trp9, Trp8b:Trp9 or Trp10(a or b)/Trp9 for quantification.

Since the prostate carcinoma forms its own basement membrane when growing invasively, it can be concluded that only cells expressing Trp8 and Trp10 are involved in this phenomenon. Thus, it can be concluded that by inhibiting the expression and/or activity of these proteins an effective therapy of cancers like PCA is provided.

Thus, the present invention also relates to a pharmaceutical composition containing a reagent which decreases or inhibits Trp8a, Trp8b, Trp10a and/or Trp10b expression or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b, and a method for preventing, treating, or ameliorating a prostate tumor, endometrial cancer (uterine carcinoma) tumor, a chorion carcinoma, cancer of the lung or melanoma, which comprises administering to a mammalian subject a

therapeutically effective amount of a reagent which decreases or inhibits Trp8a, Trp8b, Trp10a and/or Trp10b expression or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b. Examples of such reagents are the above described antisense RNAs, ribozymes or inhibitors, e.g. specific antibodies. Furthermore, peptides, which inhibit or modulate the biological function of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b may be useful as therapeutical reagents. For example, these peptides can be obtained by screening combinatorial phage display libraries (Cosmix, Braunschweig, Germany) as described by Rottgen, P. and Collins, J. (*Gene* (1995) 164 (2): 243-250). Furthermore, antigenic epitopes of the Trp8 and Trp10 proteins can be identified by the expression of recombinant Trp8 and Trp10 epitope libraries in *E. coli* (Marquart, A. & Flockerzi, V., *FEBS Lett.* 407 (1997), 137-140; Trost, C., et al., *FEBS Lett.* 451 (1999) 257-263 and the consecutive screening of these libraries with serum of patients with cancer of the prostate or of the endometrium. Those Trp8 and Trp10 epitopes which are immunogenic and which lead to the formation of antibodies in the serum of the patients can be then be used as Trp8 or Trp10 derived peptide vaccines for immune interventions against cancer cells which express Trp8 or Trp10. Alternatively to the *E. coli* expression system, Trp8 or Trp10 or epitopes of Trp8 and Trp10 can be expressed in mammalian cell lines such as human embryonic kidney (Hek 293) cells (American Type Culture Collection, ATCC CRL 1573).

Finally, compounds useful for therapy of the above described diseases comprise compounds which act as antagonists or agonists on the ion channels Trp8, Trp9 and Trp10. It could be shown that Trp8 is a highly calcium selective ion channel which in the presence of monovalent (namely sodium) and divalent ions (namely calcium) is only permeable for calcium ions (see Example 4, below, and Figures 3A, C, E). Under physiological conditions, Trp8 is a calcium selective channel exhibiting large inward currents. This very large conductance of Trp8 channels (as wells as Trp9 and Trp10a/b channels) is useful to establish systems for screening pharmacological compounds interacting with Trp-channels including high throughput screening systems. Useful high throughput screening systems are well known to the person skilled in the art and include, e.g., the use of cell lines stably or transiently transfected with DNA sequences encoding Trp8, Trp9 and Trp10 channels in assays to detect calcium signaling in biological systems. Such systems include assays based on Ca-sensitive dyes such as aequorin, apoaequorin, Fura-2, Fluo-3 and Indo-1.

Accordingly, the present invention also relates to a method for identifying compounds which act as agonists or antagonists on the ion channels Trp8, Trp9 and/or Trp10, said method comprising contacting a test compound with the ion channel Trp8, Trp9 and/or Trp10, preferably by using a system based on cells stably or transiently transfected with DNA sequences encoding Trp8, Trp9 and/or Trp10, and determining whether said test compound affects the calcium uptake.

For administration the above described reagents are preferably combined with suitable pharmaceutical carriers. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Such carriers can be formulated by conventional methods and can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g. by intravenous, intraperitoneal, subcutaneous, intramuscular, topical or intradermal administration. The route of administration, of course, depends on the nature of the tumor and the kind of compound contained in the pharmaceutical composition. The dosage regimen will be determined by the attending physician and other clinical factors. As is well known in the medical arts, dosages for any one patient depends on many factors, including the patient's size, body surface area, age, sex, the particular compound to be administered, time and route of administration, the kind and stage of the tumor, general health and other drugs being administered concurrently.

The delivery of the antisense RNAs or ribozymes of the invention can be achieved by direct application or, preferably, by using a recombinant expression vector such as a chimeric virus containing these compounds or a colloidal dispersion system. By delivering these nucleic acids to the desired target, the intracellular expression of Trp8a, Trp8b, Trp10a and/or Trp10b and, thus, the level of Trp8a, Trp8b, Trp10a and/or Trp10b can be decreased resulting in the inhibition of the negative effects of Trp8a, Trp8b, Trp10a and/or Trp10b, e.g. as regards the metastasis formation of PCA.

Direct application to the target site can be performed, e.g., by ballistic delivery, as a colloidal dispersion system or by catheter to a site in artery. The colloidal dispersion systems which can be used for delivery of the above nucleic acids include macromolecule complexes, nanocapsules, microspheres, beads and lipid-based systems including oil-in-water emulsions

(mixed), micelles, liposomes and lipoplexes, The preferred colloidal system is a liposome. The composition of the liposome is usually a combination of phospholipids and steroids, especially cholesterol. The skilled person is in a position to select such liposomes which are suitable for the delivery of the desired nucleic acid molecule. Organ-specific or cell-specific liposomes can be used in order to achieve delivery only to the desired tumor. The targeting of liposomes can be carried out by the person skilled in the art by applying commonly known methods. This targeting includes passive targeting (utilizing the natural tendency of the liposomes to distribute to cells of the RES in organs which contain sinusoidal capillaries) or active targeting (for example by coupling the liposome to a specific ligand, e.g., an antibody, a receptor, sugar, glycolipid, protein etc., by well known methods). In the present invention monoclonal antibodies are preferably used to target liposomes to specific tumors via specific cell-surface ligands.

Preferred recombinant vectors useful for gene therapy are viral vectors, e.g. adenovirus, herpes virus, vaccinia, or, more preferably, an RNA virus such as a Retrovirus. Even more preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of such retroviral vectors which can be used in the present invention are: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV) and Rous sarcoma virus (RSV). Most preferably, a non-human primate retroviral vector is employed, such as the gibbon ape leukemia virus (GaLV), providing a broader host range compared to murine vectors. Since recombinant retroviruses are defective, assistance is required in order to produce infectious particles. Such assistance can be provided, e.g., by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. Suitable helper cell lines are well known to those skilled in the art. Said vectors can additionally contain a gene encoding a selectable marker so that the transduced cells can be identified. Moreover, the retroviral vectors can be modified in such a way that they become target specific. This can be achieved, e.g., by inserting a polynucleotide encoding a sugar, a glycolipid, or a protein, preferably an antibody. Those skilled in the art know additional methods for generating target specific vectors. Further suitable vectors and methods for in vitro- or in vivo-gene therapy are described in the literature and are known to the persons skilled in the art; see, e.g., WO 94/29469 or WO 97/00957.

In order to achieve expression only in the target organ, i.e. tumor to be treated, the nucleic acids encoding, e.g. an antisense RNA or ribozyme can also be operably linked to a tissue specific promoter and used for gene therapy. Such promoters are well known to those skilled in the art (see e.g. Zimmermann et al., (1994) *Neuron* 12, 11-24; Vidal et al.; (1990) *EMBO J.* 9, 833-840; Mayford et al., (1995), *Cell* 81, 891-904; Pinkert et al., (1987) *Genes & Dev.* 1, 268-76).

For use in the diagnostic research discussed above, kits are also provided by the present invention. Such kits are useful for the detection of a target cellular component, which is Trp8a, Trp8b, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts, wherein the presence or an increased concentration of Trp8a, Trp8b, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts is indicative for a prostate tumor, endometrial cancer, melanoma, chorion carcinoma or cancer of the lung, said kit comprising a probe for detection of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts. The probe can be detectably labeled. Such probe may be a specific antibody or specific oligonucleotide. In a preferred embodiment, said kit contains an anti-Trp8a-, anti-Trp8b-, anti-Trp9-, anti-Trp10a-and/or anti-Trp10b-antibody and allows said diagnosis, e.g., by ELISA and contains the antibody bound to a solid support, for example, a polystyrene microtiter dish or nitrocellulose paper, using techniques known in the art. Alternatively, said kits are based on a RIA and contain said antibody marked with a radioactive isotope. In a preferred embodiment of the kit of the invention the antibody is labeled with enzymes, fluorescent compounds, luminescent compounds, ferromagnetic probes or radioactive compounds. The kit of the invention may comprise one or more containers filled with, for example, one or more probes of the invention. Associated with container (s) of the kit can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

EXAMPLES

The following Examples are intended to illustrate, but not to limit the invention. While such Examples are typical of those that might be used, other methods known to those skilled in the art may alternatively be utilized.

Example 1: Materials and Methods

(A) Isolation of cDNA clones and Northern blot analysis

Total RNA was isolated from human placenta and prostate using standard techniques. Isolation of mRNA was performed with poly (A)⁺RNA - spin columns (New England Biolabs, Beverly, USA) according to the instructions of the manufacturer. Poly (a)⁺RNA was reverse transcribed using the cDNA choice system (Gibco-BRL, Rockville, USA) and subcloned in λ -Zap phages (Stratagene, La Jolla, USA). An human expressed sequence tag (GenBank accession number 1404042) was used to screen an oligo d(T) primed human placenta cDNA library. Several cDNA clones were identified and isolated. Additional cDNA clones were isolated from two specifically primed cDNA libraries using primers 5'-gca tag gaa ggg aca ggt gg-3' and 5'-gag agt cga ggt cag tgg tcc-3'.

cDNA clones were sequenced using a thermocycler (PE Applied Biosystems, USA) and Thermo Sequenase (Amersham Pharmacia Biotech Europe, Freiburg, Germany). DNA sequences were analyzed with an automated sequencer (Licor, Lincoln, USA).

For Northern blot analysis 5 μ g human poly (A)⁺ RNA from human placenta or prostate were separated by electrophoresis on 0.8 % agarose gels. Poly (A)⁺ RNA was transferred to Hybond N nylon membranes (Amersham Pharmacia Biotech Europe, Freiburg, Germany). The membranes were hybridized in the presence of 50 % formamide at 42°C over night. DNA probes were labelled using [α^{32} P]dCTP and the „ready prime,, labelling kit (Amersham Pharmacia Biotech Europe, Freiburg, Germany). Commercial Northern blots were hybridized according to the distributors instructions (Clontech, Palo Alto, USA).

(B) Construction of expression plasmids and transfection of HEK 293 cells

Lipofections were carried out with the recombinant dicistronic eucaryotic expression plasmid pdiTRP8 containing the cDNA of Trp8b under the control of the chicken β -actin promoter followed by an internal ribosome entry site (IRES) and the cDNA of the green fluorescent protein (GFP). To obtain pdiTRP8 carrying the entire protein coding regions of TRP8b and

the GFP (Prasher, D.C. et al. (1992), Gene 111, 229-233), the 5' and 3'-untranslated sequences of the TRP8b cDNA were removed, the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) Nucleic Acids Research 15, 8125-8148) was introduced immediately 5' of the translation initiation codon and the resulting cDNA was subcloned into the pCAGGS vector (Niwa, H., Yamamura, K. and Miyazaki, J (1991), Gene 8, 193-199) downstream of the chicken β -actin promoter. The IRES derived from encephalomyocarditis virus (Kim, D.G., Kang, H.M., Jang, S.K. and Shin H.S. (1992) Mol.Cell.Biol. 12, 3636-3643) followed by the GFP cDNA containing a Ser65Thr mutation (Heim, R., Cubitt, A.B., Tsien, R.Y. (1995) Nature 373, 663-664) was then cloned 3' to the TRP8b cDNA. The IRES sequence allows the simultaneous translation of TRP8b and GFP from one transcript. Thus, transfected cells can be detected unequivocally by the development of green fluorescence.

For monitoring of the intracellular Ca^{2+} concentration human embryonic kidney (HEK 293) cells were cotransfected with the pcDNA3-TRP8b vector and the pcDNA3-GFPvector in a molar ratio of 4 : 1 in the presence of lipofectamine (Quiagen, Hilden, Germany). To obtain pcDNA3-TRP8b the entire protein coding region of TRP8b including the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) Nucleic Acids Research 15, 8125-8148) was subcloned into the pcDNA3 vector (Invitrogen, Groningen, Netherlands). Calcium monitoring and patch clamp experiments were carried out two days and one day after transfection, respectively.

(C) Chromosomal localization of the Trp8 gene

The chromosomal localization of the human TRP8 gene was performed using NIGMS somatic hybrid mapping panel No.2 (Coriell Institute, Camden, NJ, USA) previously described (Drwina, H.L., Toji, L.H., Kim, C.H., Greene, A.E., Mulivor, R.A. (1993) Genomics 16, 311-314; Dubois, B.L. and Naylor, S.L. (1993) Genomics 16, 315-319).

(D) In Vitro Translation, glutathione - sepharose and calmodulin agarose binding assay

N- and C-terminal Trp8-fragments were subcloned into the pGEX-4T2 vector (Amersham Pharmacia Europe, Freiburg, Germany) resulting in glutathione-S-transferase (GST)-Trp8 fusion constructs (Fig. 4). The GST-TRP8-fusion proteins were expressed in E. coli BL 21 cells and purified using glutathione - sepharose beads (Amersham Pharmacia Biotech Europe, Freiburg, Germany).

In vitro translation of human Trp8 cDNA and *Xenopus laevis* calmodulin cDNA (Davis, T.N. and Thorner, J. Proc.Natl.Acad.Sci. USA 86, 7909-7913.) was performed in the presence of ^{35}S -methionine using the TNT coupled transcription/translation kit (Promega, Madison, USA). Translation products were purified by gel filtration (Sephadex G50, Amersham Pharmacia Biotech Europe, Freiburg, Germany) and equal amounts of ^{35}S labeled probes were incubated for 2 h with glutathione beads bound to GST - Trp8 or calmodulin - agarose (Calbiochem) in 50 mM Tris-HCl, pH 7.4, 0.1 % Triton X-100, 150 mM NaCl in the presence of 1 mM Ca^{2+} or 2 mM EGTA. After three washes, bound proteins were eluted with SDS sample buffer, fractionated by SDS-PAGE and ^{35}S labeled proteins were detected using a Phosphor Imager (Fujifilm, Tokyo, Japan).

(E) Calcium measurements

The intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) was determined by dual wavelength fura-2 fluorescence ratio measurements (Tsien, R.Y. (1988) Trends Neurosci. 11, 419-424) using a digital imaging system (T.I.L.L. Photonics, Planegg, Germany). HEK cells were grown in minimal essential medium in the presence of 10 % fetal calf serum and cotransfected with the pcDNA3-TRP8b vector and the pCDNA3-GFPvector as described above (B). Transfected cells were detected by development of green fluorescence. The cells were loaded with $4\mu\text{M}$ fura-2/AM (Molecular Probes, Oregon, USA) for one hour. After loading the cells were rinsed 3 times with buffer B1 (10 mM Hepes, 115 mM NaCl, 2 mM MgCl_2 , 5mM KCl, pH 7.4) and the $[\text{Ca}^{2+}]_i$ was calculated from the fluorescence ratios obtained at 340 and 380 nm excitation wavelengths as described (Garcia, D.E., Cavalié, A. and Lux, H.D. (1994) J. Neurosci 14, 545-553).

(F) Electrophysiological recordings

HEK cells were transfected with the eucaryotic expression plasmid pdiTRP8 described in (B) and electrophysiological recordings were carried out one day after transfection. Single cells were voltage clamped in the whole cell mode of the patch clamp technique as described (Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85-100; Philipp, S., Cavalié, A., Freichel, M., Wissenbach, U., Zimmer, S., Trost, C., Marquart, A., Murakami, M. and Flockerzi, V. (1996) EMBO J. 6166-6171). The pipette solution contained (mM): 140 aspartic acid, 10 EGTA, 10 NaCl, 1 MgCl_2 , 10 Hepes (pH 7.2 with CsOH) or 125 CsCl, 10 EGTA, 4 CaCl_2 10 Hepes (pH 7,2 with CsOH). The bath solution contained (mM): 100 NaCl, 10 CsCl, 2 MgCl_2 , 50 mannitol, 10 glucose, 20

Hepes (pH 7,4 with CsOH) and 2 CaCl₂, or no added CaCl₂ (-Ca²⁺ solution). Divalent free bath solution contained (mM): 110 N-methyl-D-glucamine (NMDG). Whole cell currents were recorded during 100 msec voltage ramps from -100 to +100 mV at varying holding potentials.

(G) In Situ Hybridization

In situ hybridizations were carried out using formalin fixed tissue slices of 6 - 8 µM thickness. The slices were hydrated and incubated in the presence of PBS buffer including 10 µg / ml proteinase K (Roche Diagnostics, Mannheim, Germany) for 0.5 h. The slices were hybridized at 37°C using biotinylated deoxy-oligonucleotides (0.5 pmol / µl) in the presence of 33 % formamide for 12 h. Furthermore the slices were several times rinsed with 2 x SSC and incubated at 25°C for 0.5 h with avidin / biotinylated horse raddish peroxidase complex (ABC, DAKO, Santa Barbara, USA). After several washes with PBS buffer the slices were incubated in the presence of biotinylated tyramid and peroxide (0.15 % w/v) for 10 min, rinsed with PBS buffer and additionally incubated with ABC complex for 0.5 h. The slices were washed with PBS buffer and incubated in the presence of DAB solution (diaminobenzidine (50µg / ml), 50 mM Tris/EDTA buffer pH 8.4, 0.15 % H₂O₂ in N,N - dimethyl-formamide; Merck, Darmstadt, Germany), The detection was stopped after 4 minutes by incubating the slides in water. Tyramid was biotinylated by incubating NHS-LC Biotin (sulfosuccinimidyl-6-(biotinimid)-hexanoat), 2.5 mg / ml; Pierce, Rockford, USA) and tyramin-HCl (0.75 mg / ml, Sigma) in 25 mM borate buffer pH 8.5 for 12 h. The tyramid solution was diluted 1 - 5 : 1000 in PBS buffer.

(H) GenBank accession numbers: TRP8a, Aj243500; TRP8b Aj243501

Example 2: Expression of TRP8 transcripts

In search of proteins distantly related to the TRP family of ion channels, an human expressed sequence tag (EST, GenBank accession number 1404042) was identified in the GenBank database using BLAST programmes (at the National Center for Biotechnology Information (NCBI); Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J.J. (1990) Mol. Biol. 5, 403-410) being slightly homologous to the VR1 gene. Several human placenta cDNA libraries were constructed and screened with this EST DNA as probe. Several full length

cDNA clones were identified and isolated. The full length cDNA clones encoded two putative proteins differing in three amino acids and were termed Trp8a and Trp8b (Fig. 1c, 2a, 7 and 8A). This finding was reproduced by isolating cDNA clones from two cDNA libraries constructed from two individual placentas. The derived protein sequence(s) comprises six transmembrane domains, a characteristic overall feature of trp channels and related proteins (Fig.: 1b). The sequence is closely related to the meanwhile published calcium uptake transport protein 1 (CaT1), isolated from rat intestine (Peng, J.B., Chen, X.Z., Berger, U.V., Vassilev, P.M., Tsukaguchi, H., Brown, E.M. and Hediger M.A.(1999) *J Biol Chem.* 6;274, 22739-22746) and to the epithelial calcium uptake channel (ECaC) isolated from rabbit kidney (Hoenderop, J.G., van der Kemp, A.W., Hartog, A., van de Graaf, S.F., van Os, C.H., Willems, P.H. and Bindels, R.J. (1999) *J Biol Chem.* 26;274, 8375-8378). Expression of Trp8a/b transcripts are detectable in human placenta, pancreas and prostate (Fig.: 5) and the size of the Northern signal (3.0 kb) corresponds with the size of the isolated full length cDNAs. In addition, a shorter transcript of 1.8 kb, probably a splice variant, is detectable in human testis. The Trp8 mRNA is not expressed in small intestine or colon (Fig.: 5) implicating that Trp8 is not the human ortholog of the rat CaT1 or rabbit ECaC proteins. To investigate whether there are other related sequences Trp8a/b derived primers (UW241, 5'-TAT GAG GGT TCA GAC TGC-3' and UW242, 5'-CAA AGT AGA TGA GGT TGC-3') were used to amplify a 105 bp fragment from human genomic DNA being 95% identical on the nucleotide level to the Trp8 sequence (data not shown). This indicates the existence of several similar sequences in humans at least at the genomic level.

Example 3: Two variants of the Trp8 protein (Trp8a and Trp8b) arise by polymorphism

Two variants of the Trp8 cDNA were isolated from human placenta (Fig.: 2A, 7 and 8A) which encoded two proteins which differ in three amino acids and were termed Trp8a and Trp8b. Trp8a/b specific primers were designed to amplify a DNA fragment of 458 bp of the Trp8 gene from genomic DNA isolated from human T-lymphocytes (primer pair: UW243, 5'-CAC CAT GTG CTG CAT CTA CC-3' and UW244, 5'-CAA TGA CAG TCA CCA GCT CC-3'). The amplification product contains a part of the sequence where the derived protein sequence of Trp8a comprises the amino acid valine and the Trp8b sequence methionine as well as a silent base pair exchange (g versus a) and an intron of 303bp (Fig.: 2.A, B). Both variants of the Trp8 genes (a,b) were amplified from genomic DNA in equal amounts indicating the existence of both variants in the human genome and therefore being not the

result of RNA editing (Fig.: 2B). The Trp8a gene can be distinguished from the Trp8b gene by cutting the genomic fragment of 458bp with the restriction enzyme Bsp1286I (Fig. 2B). Using human genomic DNA isolated from blood of twelve human subjects as template, the 458bp fragment was amplified and restricted with BSP1286I. In eleven of the tested subjects only the Trp8b gene is detectable, while one subject (7) contains Trp8a and Trp8b genes (Fig.: 2D). These implicates that the two Trp8 variants arise by polymorphism and do not represent individual genes. Using Trp8 specific primers and chromosomal DNA as template, the Trp8 locus is detectable on chromosome 7 (Fig.: 2C).

Example 4: Trp8b is a calcium permeable channel

The protein coding sequence of the Trp8b cDNA was subcloned into pcDNA3 vector (Invitrogen, Groningen, Netherlands) under the control of the cytomegalovirus promotor (CMV). Human embryonic kidney (HEK 293) cells were cotransfected with the Trp8b pcDNA3 construct (pcDNA3-Trp8b vector) and the pcDNA3-GFPvector encoding the green fluorescent protein (GFP) in 4:1 ratio. The Trp8b cDNA and the cDNA of the reporter, GFP, was transiently expressed in human embryonic kidney (HEK 293) cells. The intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and changes of $[\text{Ca}^{2+}]_i$ were determined by dual wavelength fura-2 fluorescence ratio measurements (Fig.: 3F) in cotransfected cells which were identified by the green fluorescence of the reporter gene GFP.

Dual wavelength fura-2 fluorescence ratio measurement is a standard procedure (e.g. in: An introduction of Molecular Neurobiology (ed. Hall, Z.W.) Sinauer Associates, Sunderland, USA (1992)) using fura-2, which is a fluorescent Ca^{2+} sensitive dye and which was designed by R.Y.Tsien (e.g. Trends Neurosci. 11, 419-424 (1988) based upon the structure of EGTA. Its fluorescence emission spectrum is altered by binding to Ca^{2+} in the physiological concentration range. In the absence of Ca^{2+} , fura-2 fluoresces most strongly at an excitation wavelength of 385 nm; when it binds Ca^{2+} , the most effective excitation wavelength shifts to 345 nm. This property is used to measure local Ca^{2+} concentrations within cells. Cells can be loaded with fura-2 esters (e.g. fura-2AM) that diffuse across cell membranes and are hydrolyzed to active fura-2 by cytosolic esterases.

In the presence of 1mM Ca^{2+} , Trp8 expressing cells typically contained more than 300 nM cytosolic Ca^{2+} , while non transfected controls contained less than 100 nM Ca^{2+} ions (Fig. 3F).

When Trp8b transfected cells were incubated without extracellular Ca^{2+} , the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) decreased to levels comparable to non transfected cells. Readdition of 1mM Ca^{2+} to the bath resulted in significant increase of the cytosolic $[\text{Ca}^{2+}]$ in Trp8b transfected cells, but not in controls (Fig.: 3F). After readdition of Ca^{2+} ions to the bath solution, the cytosolic Ca^{2+} concentration remains on a high steady state level in the Trp8b transfected cells.

Example 5: Trp8 expressing cells show calcium selective inward currents

To characterize in detail the electrophysiological properties of TRP8, TRP8 and GFP were coexpressed in HEK293 cells using the dicistronic expression vector pdiTRP8 and measured currents using the patch clamp technique in the whole cell mode (Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) *Pflugers Arch.*, 391, 85-100).

The eucaryotic expression plasmid pdiTRP8 contains the cDNA of Trp8b under the control of the chicken β -actin promotor followed by an internal ribosome entry side (IRES) and the cDNA of the green fluorescent protein (GFP). To obtain pdiTRP8 carrying the entire protein coding regions of TRP8b and the GFP (Prasher, D.C. et al. (1992), *Gene* 111, 229-233), the 5' and 3'-untranslated sequences of the TRP8b cDNA were removed, the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) *Nucleic Acids Research* 15, 8125-8148) was introduced immediately 5' of the translation initiation codon and the resulting cDNA was subcloned into the pCAGGS vector (Niwa, H., Yamamura, K. and Miyazaki, J (1991), *Gene* 8, 193-199) downstream of the chicken β -actin promotor. The IRES derived from encephalomyocarditis virus (Kim, D.G., Kang, H.M., Jang, S.K. and Shin H.S. (1992) *Mol.Cell.Biol.* 12, 3636-3643) followed by the GFP cDNA containing a Ser65Thr mutation (Heim, R., Cubitt, A.B., Tsien, R.Y. (1995) *Nature* 373, 663-664) was then cloned 3' to the TRP8b cDNA. The IRES sequence allows the simultaneous translation of TRP8b and GFP from one transcript. Thus, transfected cells can be detected unequivocally by the development of green fluorescence.

In the presence of 2 mM external calcium, Trp8b transfected HEK cells show inwardly rectifying currents, the size of which depends on the level of intracellular calcium and the electrochemical driving force. The resting membrane potential was held either at -40 mV, or, to lower the driving force for calcium influx in between pulses, at + 70 mV. Current traces

were recorded in response to voltage ramps from -100 to $+100$ mV, that were applied every second. To monitor inward and outward currents over time, we analyzed the current size at -80 and $+80$ mV of the ramps. Figure 3A shows a representative trace of the current at -80 mV over time. Both at a holding potential of -40 mV or at $+70$ mV, the currents are significantly larger than in cells transfected with only the GFP containing vector (Fig.: 3E). Interestingly, after changing to a positive holding potential, current size in Trp8 transfected cells slowly increases and reaches steady state after approximately 70 seconds (Fig.: 3A). To determine the selectivity of the induced currents, we then perfused the cells with solutions that either contain no sodium, no added Ca^{2+} (Fig. 3A, C) or a sodium containing, but divalent ion free bath solution. To control for the effect of the solution change alone, we also perfused with normal bath (see puff in Fig. 3A). While removal of external Ca^{2+} completely abolishes the trp 8 induced currents - the remaining current being identical in size and shape to the control (Fig.: 3A, C, E), removal of external sodium has no effect (Fig.: 3E). An important hallmark of calcium selective channels (e.g. Vennekens, R., Hoenderop, G.J., Prenen, J., Stuijver, M., Willems, PHGM, Droogmans, G., Nilius, B. and Bindels, R.J.M (1999) *J. Biol. Chem.* 275, 3963-3969), is their ability to conduct sodium only if all external divalent ions, namely Ca^{2+} and magnesium are removed. To test whether the trp 8 channel conforms with this phenomenon normal bath solution was switched to a solution containing only sodium and 1 mM EGTA. As can be seen in Figure 3B and D, Trp8 transfected cells can now conduct very large sodium currents. Interestingly, immediately after the solution change, the currents first become smaller before increasing rapidly, indicating that the pore may initially still be blocked by calcium a phenomenon usually called anomalous mole fraction behaviour (Warnat, J., Philipp, S., Zimmer, S., Flockerzi, V., and Cavalié A. (1999) *J. Physiol. (Lond)* 518, 631-638). The measured outward currents of Trp8 transfected cells in normal bath solution are not significantly different from non-transfected control cells or cells which only express the reporter gene GFP. As the removal of external Ca^{2+} abolishes the Trp8 specific current, the remaining current was subtracted from the current before the solution change to obtain the uncontaminated Trp8 conductance (see inset in Fig.: 3C). As expected from the given ionic conditions (high EGTA inside, 2 mM Ca^{2+} outside), the current-voltage relationship now shows prominent inward rectification with little to no outward current.

Both the time course of the development of Trp8 currents and the size of the currents depend on the frequency of stimulation (data not shown), the internal and external Ca^{2+} concentration

and the resting membrane potential, suggesting that Trp8 calcium conductance is intricately regulated by a Ca^{2+} mediated feedback mechanisms.

Example 6: Ca^{2+} / calmodulin binds to the C-terminus of the Trp8 protein

To test whether calmodulin, a prime mediator of calcium regulated feedback, is involved, first it was investigated biochemically whether Trp8 protein can bind calmodulin. Trp8 cDNA was in vitro translated in the presence of ^{35}S -methionine and the product incubated with calmodulin-agarose beads. After several washes either in the presence or absence of Ca^{2+} , the beads were incubated in Laemmli buffer and subjected to SDS-polyacrylamide gel electrophoresis. In the presence of Ca^{2+} (1mM), but not in the absence of Ca^{2+} , Trp8 protein binds to calmodulin (Fig.: 4B).

To narrow down the binding site, two approaches were undertaken: Firstly, GST-TRP8 fusion proteins of various intracellular domains of Trp8 were constructed, expressed in *E. coli* and bound to glutathione sepharose beads. These beads were then incubated with in vitro translated ^{35}S - labeled calmodulin, washed and subjected to gel electrophoresis. Secondly, truncated versions of in vitro translated Trp8 protein were used in the above described binding to calmodulin-agarose. As shown in Figure 4A, and C, fusion proteins of the N-terminal region (N1, N2) of Trp8 did not bind calmodulin, while C-terminal fragments (C1, C2, C3, C4) showed calmodulin binding in the presence of calcium (for localization of fragments within the entire Trp8 protein see Fig. 4C). Accordingly, a truncated version of in vitro translated Trp8, which lacks the C-terminal 32 amino acid residues did not bind to calmodulin-agarose (4B). We have restricted the calmodulin binding site to amino acid residues 691 to 711 of the Trp8 protein. This calmodulin binding site does not resemble the typical conserved IQ - motif of conventional myosins, but has limited sequence homology to the calcium dependent calmodulin binding site 1 of the transient receptor potential like (trpl) protein of *Drosophila melanogaster* (Warr and Kelly, 1996) with several charged amino acid residues conserved. The sequence of the calmodulin binding site of the Trp8 protein resembles a putative amphipathic α -helical wheel structure with a charged and a hydrophobic site according to a model proposed by Erickson-Vitanen and De Grado (1987, *Methods Enzymol.* 139, 455-478.).

Example 7: Expression of Trp8 transcripts in human placenta and pancreas

Several slides from a human placenta of a ten week old abort were used for in situ hybridization experiments. The in situ hybridization experiments revealed expression of Trp8 transcripts in human placenta (Fig.: 5B). Expression was detectable in trophoblasts and syncytiotrophoblasts of the placenta, but not in Langerhans cells.

Trp8 transcripts are detectable in human pancreas (Fig.: 5A). Therefore Trp8 probes were hybridized to tissue sections of human pancreas. The pancreatic tissues were removed from patients with pancreas cancer. Trp8 expression is detectable in pancreatic acinar cells, but not in Langerhans islets (Fig.: 5C). No Trp8 expression was found in regions of pancreatic carcinomas (data not shown).

Furthermore, the Trp8 cDNA is not detectable in human colon nor in human kidney by in situ hybridization as well as by Northern analysis (Fig.: 5A, D). The Northern results taken together with the in situ expression data indicate that the Trp8 protein is not the human ortholog of the CaT1 and ECaC channels cloned from rat intestine (Peng, J.B., Chen, X.Z., Berger, U.V., Vassilev, P.M., Tsukaguchi, H., Brown, E.M. and Hediger M.A.(1999) J Biol Chem. 6;274, 22739-22746) and from rabbit kidney (Hoenderop, J.G., van der Kemp, A.W., Hartog, A., van de Graaf, S.F., van Os, C.H., Willems, P.H. and Bindels, R.J. (1999) J Biol Chem. 26;274, 8375-8378), respectively. Trp8 is unlikely to represent the human version of CaT1 as its expression is undetectable in the small intestine and colon tissues where CaT1 is abundantly expressed. If, however, Trp8 is the human version of rat CaT1, a second gene product appears to be required for Ca^{2+} uptake in human small intestine and colon attributed to CaT1 in rat small intestine and colon.

Example 8: Differential expression of Trp8 transcripts in benign and malignant tissue of the prostate

The Trp8 transcripts are expressed in human prostate as shown by hybridization of a Trp8 probe to a commercial Northern blot (Clontech, Palo Alto, USA) (Fig.: 5A). Trp8 transcripts were not detectable by Northern blot analysis using pooled mRNA of patients with benign prostatic hyperplasia (BPH) (Fig.: 5A, prostate*). To examine Trp8 expression on the cellular

level, sections of prostate tissues were hybridized using Trp8 specific cDNA probes (Table 3). Expression of Trp8 transcripts is not detectable in normal prostate (n = 3), benign hyperplasia (BPH, n = 15) or prostatic intraepithelial neoplasia (PIN, n = 9) (Fig.: 6A, C, E). Trp8 transcripts were only detectable in prostate carcinoma (PCA), although with different expression levels. Low expression levels were found in primary carcinomas (2 - 10 % of the carcinoma cells, n = 8) (Fig.: 7B). Much stronger expression was detectable in rezidive carcinoma (10 - 60 %) (Fig.: 7D, n = 6) and metastases of the prostate (60 - 90 %, n = 4) (Fig.: 7F). Thus it has to be concluded that the commercial Northern blot used in Fig.: 5A contains not only normal prostate mRNA as indicated by the distributor. According to the distributors instructions the prostate mRNA used for this Northern blot was collected from 15 human subjects in the range of 14 to 60 years of age. This prostate tissue was not examined by pathologic means. Since Trp8 expression is not detectable in normal or benign prostate, this finding implicates that the mRNA used for this Northern blot was extracted in part from prostatic carcinoma tissue. To summarize, Trp8 expression is only detectable in malign prostate and, thus, the Trp8 cDNA is a marker for prostate carcinoma. The results are summarized in Table 4.

Table 3

Trp8 probes used for in situ hybridization:

Probes (antisense)

- 1.) 5' TCCGCTGCCGGTTGAGATCTTGCC 3'
- 2.) 5' CTTGCTCCATAGGCAGAGAATTAG 3'
- 3.) 5' ATCCTCAGAGCCCCGGGTGTGGAA3'

Controls (sense)

- 1.) 5' GGCAAGATCTCAACCGGCAGCGGA 3'
- 2.) 5' CTAATTCTCTGCCTATGGAGCAAG 3'
- 3.) 5' TTCCACACCCGGGGCTCTGAGGAT 3'

Table 4

Prostate	total	negative	positive
normal	3	3	0
BPH	15	15	0
PIN	9	9	0

carcinoma

18

1

17

(B) Differential expression of Trp8 transcripts in benign and malign tissue of the uterus

Moreover it could be shown that Trp8 is expressed in endometrial cancer (also called cancer of the uterus, to be distinguished from uterine sarcoma or cancer of the cervix) whereas no expression was observed in normal uterus tissue. Thus, Trp8 also is a specific marker for the diagnosis of the above cancer (Fig. 12).

Example 9: Characterization of Trp9

The complete protein coding sequence of Trp9 was determined (Fig. 9). Trp 9 transcripts are predominantly expressed in the human prostate and in human colon. As it could be shown by Northern blot analysis, there is no difference of the expression of TRP9 in benign prostatic hyperplasia (BPH, Fig. 13, upper panel left) or prostate carcinoma (Fig. 13, upper panel right). However, Trp9 is useful as a reference marker for prostate carcinoma, i.e. can be used for quantifying the expression level of Trp8. The ratio of the expression of Trp8:Trp9 in patients and healthy individuals is useful for the development of a quantitative assay.

Example 10: Characterization of Trp10

The complete protein coding sequence of TRP10 (a and b) was determined by biocomputing (Fig. 10 and 11). Using a 235 bp fragment of the Trp10 cDNA as probe in Northern blot analysis TRP10 transcripts could only be detected in mRNA isolated from individuals with prostate cancer (Fig. 13, bottom panel) but not in mRNA isolated from benign tissue of the prostate (prostate BPH) nor in mRNA isolated from heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. The 235 bp cDNA fragment of the Trp10 cDNA was amplified using the primer pair UW248 5'-ACA GCT GCT GGT CTA TTC C-3' and UW249 5'-TAT

GTG CCT TGG TTT GTA CC-3' and prostate cDNA as template. In summary, Trp10a and Trp10b, like TRP8 are also expressed in malignant prostate tissue. So far, its expression could not be observed in any other tissue examined (see above). Thus, Trp 10a and Trp10b are also useful markers which are specific for malignant prostate tissue.

Furthermore, database searches in public databases of the national center for biological information (NCBI) revealed the existence of several expressed sequence tags (EST clones) being in part identical to the Trp10 sequence. These EST clones were originally isolated from cancer tissues of lung, placenta, prostate and from melanoma. These clones include the clones with the following accession numbers: BE274448, BE408880, BE207083, BE791173, AI671853, BE390627. The results demonstrate that cancer cells of these tissues express Trp10 related transcripts whereas no expression of Trp10 transcripts in the corresponding healthy tissues are detectable (Figure 13). Furthermore, it could be shown that in cancer cells of melanoma and prostate cancer Trp10 transcripts are expressed as shown by in situ hybridizations using 4 antisense probes (Figure 14A – E and 13K-O and Table 2, above). Furthermore, it could clearly be shown that cancer cells of these tissues expressing Trp10 transcripts also express Trp10-antisense transcripts as shown in Figure 14F-J, Figure 14P-R and Figure 14T by in situ hybridizations using 4 sense probes (Table 2, above). The in situ hybridization experiments demonstrate that detection of a subset of cancer cells derived from carcinoma of lung, placenta, prostate and melanoma is feasible using antisense as well as sense probes complementary to Trp10 transcripts or complementary to Trp10-antisense transcripts, respectively.

The foregoing is meant to illustrate but not to limit the scope of the invention. The person skilled in the art can readily envision and produce further embodiment, based on the above teachings, without undue experimentation.

What Is claimed Is:

1. An isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b or a protein exhibiting biological properties of Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being selected from the group consisting of
 - (a) a nucleic acid molecule encoding a protein that comprises the amino acid sequence depicted in Figure 7, 8A, 9, 10 or 11;
 - (b) a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9, 10 or 11;
 - (c) a nucleic acid molecule included in DSMZ Deposit No. DSM 13579, DSM 13580, DSM 13584, DSM 13581 or DSM....;
 - (d) a nucleic acid molecule which hybridizes to a nucleic acid molecule specified in (a) to (c);
 - (e) a nucleic acid molecule the nucleic acid sequence of which deviates from the nucleic sequences specified in (a) to (d) due to the degeneration of the genetic code; and
 - (f) a nucleic acid molecule, which represents a fragment, derivative or allelic variation of a nucleic acid sequence specified in (a) to (e).
2. A recombinant vector containing the nucleic acid molecule of claim 1
3. The recombinant vector of claim 2 wherein the nucleic acid molecule is operatively linked to regulatory elements allowing transcription and synthesis of a translatable RNA in prokaryotic and/or eukaryotic host cells.
4. A recombinant host cell which contains the recombinant vector of claim 3.
5. The recombinant host cell of claim 4, which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.
6. An isolated protein exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b which is encoded by a nucleic acid molecule of claim 1.
7. A recombinant host cell that expresses the isolated protein of claim 6.

8. A method of making an isolated protein exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b comprising:
(a) culturing the recombinant host cell of claim 6 under conditions such that said protein is expressed; and
(b) recovering said protein.
9. The protein produced by the method of claim 8.
10. An antisense RNA sequence characterized in that it is complementary to an mRNA transcribed from a nucleic acid molecule of claim 1 or a part thereof and can selectively bind to said mRNA or part thereof, said sequence being capable of inhibiting the synthesis of the protein encoded by said nucleic acid molecule.
11. A ribozyme characterized in that it is complementary to an mRNA transcribed from a nucleic acid molecule of claim 1 or a part thereof and can selectively bind to and cleave said mRNA or part thereof, thus inhibiting the synthesis of the protein encoded by said nucleic acid molecule.
12. An inhibitor characterized in that it can suppress the activity of the protein of claim 6.
13. A method for diagnosing a prostate carcinoma which comprises contacting a target sample suspected to contain the protein Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA with a reagent which reacts with Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA and detecting Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA.
14. The method of claim 13, wherein the reagent is a nucleic acid.
15. The method of claim 13, wherein the reagent is an antibody.
16. The method of claim 13, wherein the reagent is detectably labeled.

17. The method of claim 16, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.
18. A method for diagnosing an endometrial cancer (carcinoma of the uterus) which comprises contacting a target sample suspected to contain the protein Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA with a reagent which reacts with Trp8a and/or Trp8b or the Trp8a and/or Trp8a and/or trp8b encoding mRNA and detecting Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA.
19. The method of claim 18, wherein the reagent is a nucleic acid.
20. The method of claim 18, wherein the reagent is an antibody.
21. The method of claim 18, wherein the reagent is detectably labeled.
22. The method of claim 21, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.
23. A method for diagnosing a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense RNA or Trp10a and/or Trp10b related antisense RNA.
24. A method for preventing, treating, or ameliorating a prostate tumor, endometrial cancer (carcinoma of the uterus) tumor, a chorion carcinoma, cancer of the lung or melanoma, which comprises administering to a mammalian subject a therapeutically effective amount of a reagent which decreases or inhibits expression of Trp8a, Trp8b, Trp10a and/or Trp10b and/or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b.
25. The method of claim 24, wherein the reagent is a nucleotide sequence comprising an antisense RNA.

26. The method of claim 24, wherein the reagent is a nucleotide sequence comprising a ribozyme.
27. The method of claim 24, wherein the reagent is an inhibitor of Trp8a, Trp8b, Trp10a and/or Trp10b.
28. The method of claim 27, wherein the reagent is an anti-Trp8a-, anti Trp8b-, anti-Trp10a- and/or anti-Trp10b antibody or a fragment thereof.
29. A diagnostic kit useful for the detection of Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts in a sample, wherein the presence of an increased concentration of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts is indicative for a prostate tumor, endometrial cancer (cancer of the uterus) tumor, a chorion carcinoma, cancer of the lung or melanoma, said kit comprising a probe for detection of Trp8a, Trp8b, Trp9, Trp10a or Trp10b or Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts.
30. The kit of claim 29, wherein the target component to be detected is Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b and the probe is an antibody.
31. A method for identifying a compound which acts as an agonist or antagonist on the ion channels Trp8, Trp9 and/or Trp10, said method comprising contacting a test compound with the ion channel Trp8, Trp9 and/or Trp10, and determining whether said test compound affects the calcium uptake.

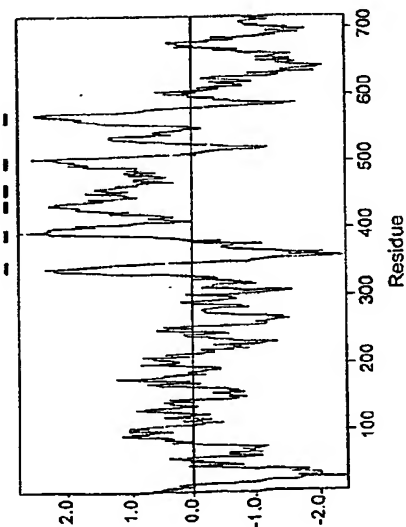
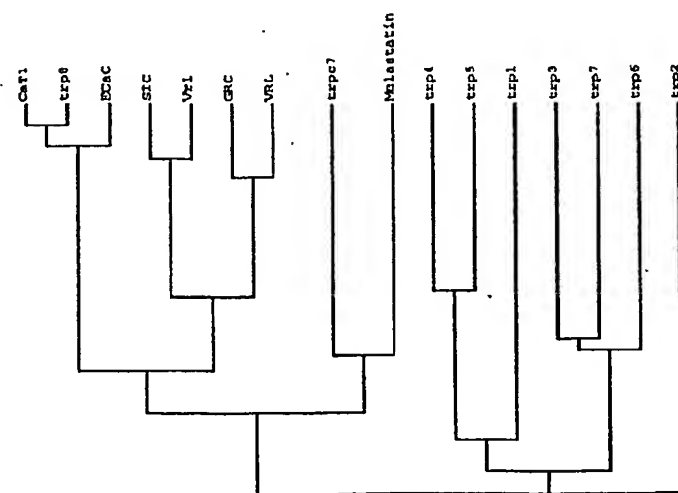
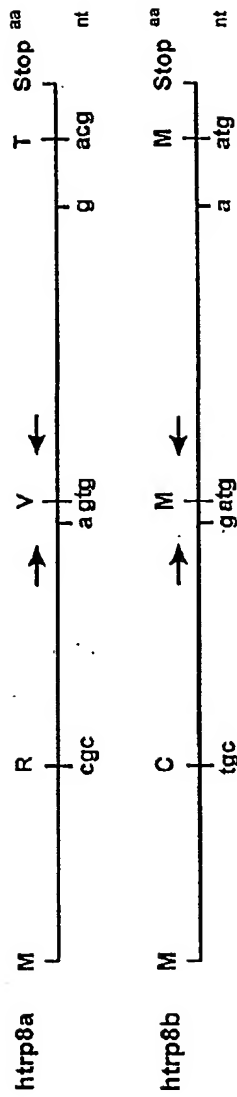
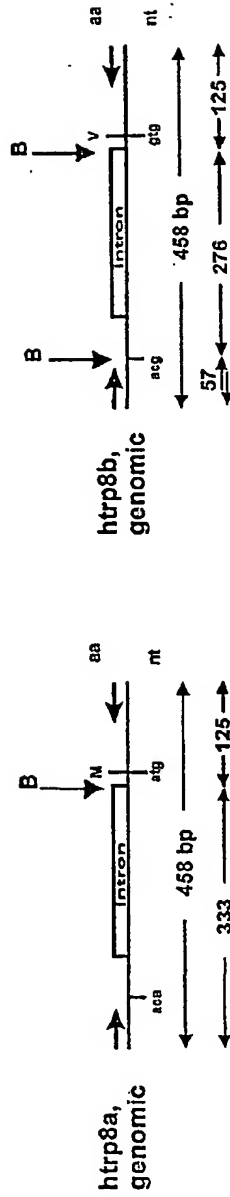
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Fig. 2

A

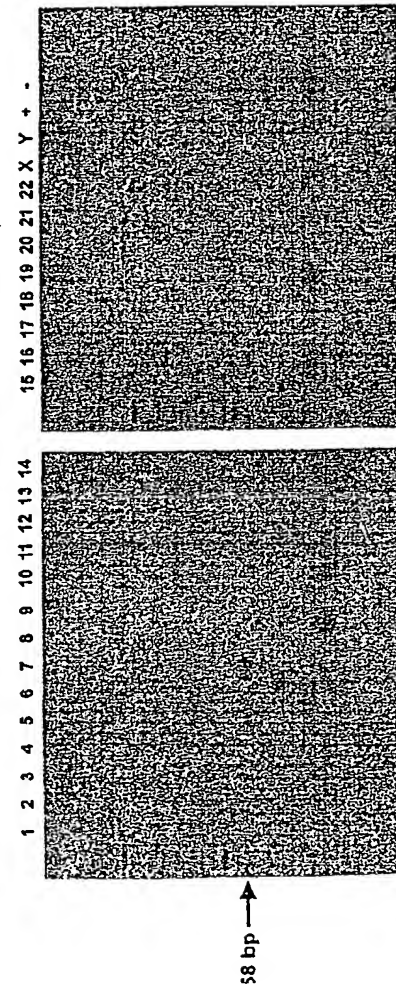


B



C

Chromosome



D

Genotype

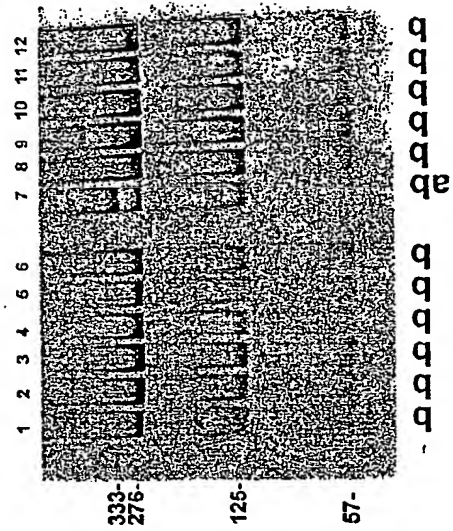


Fig. 3

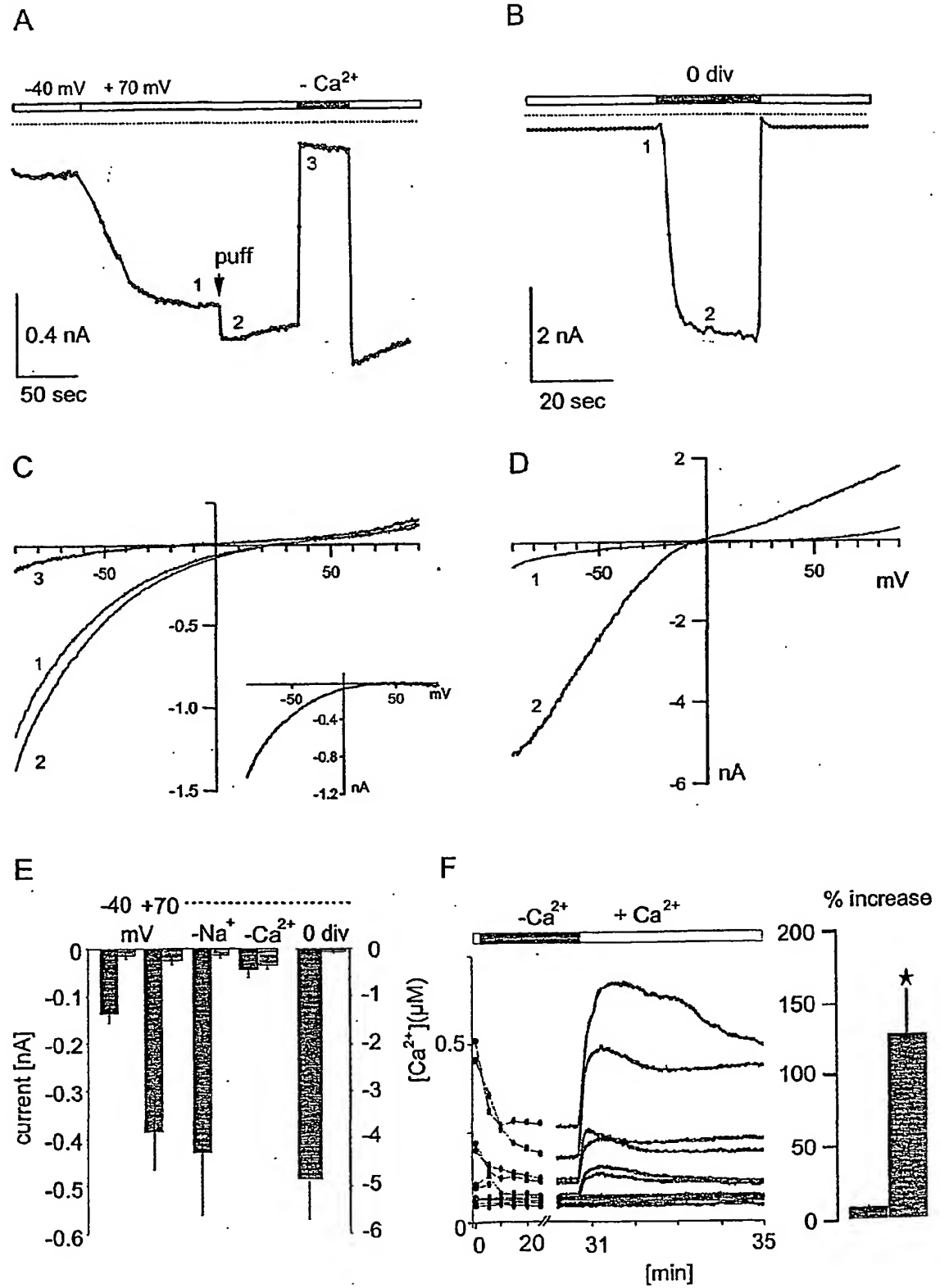


Fig. 4

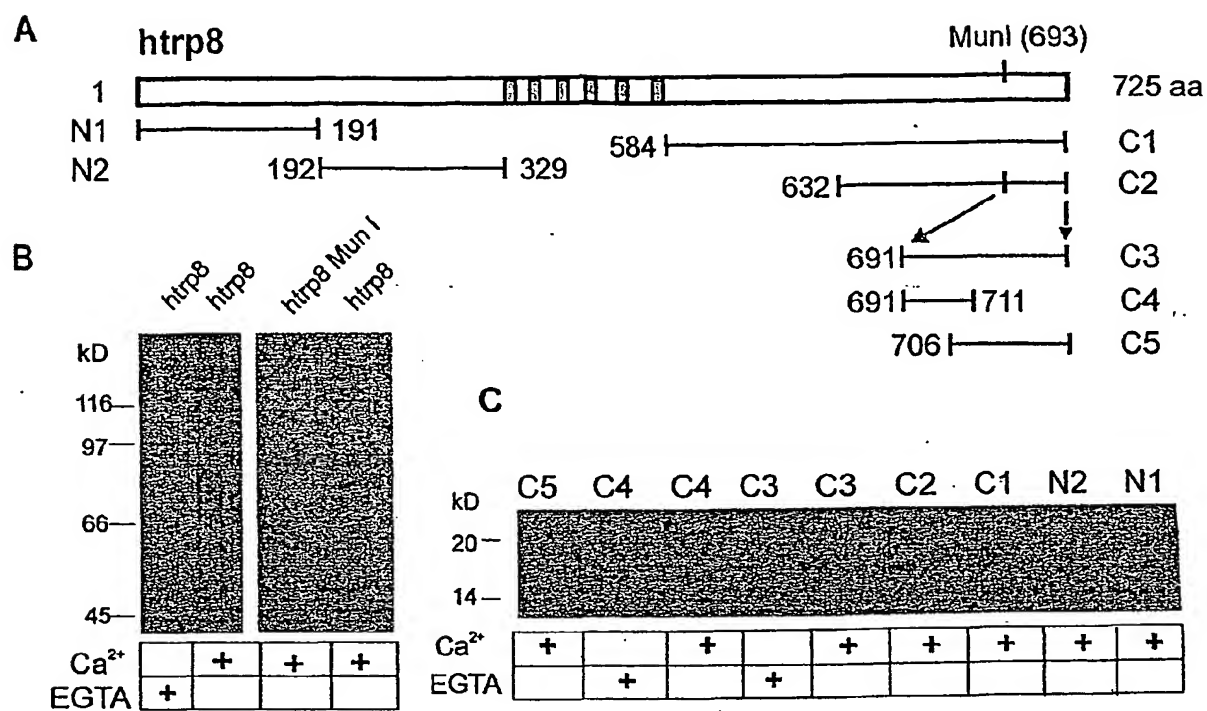


Fig. 5

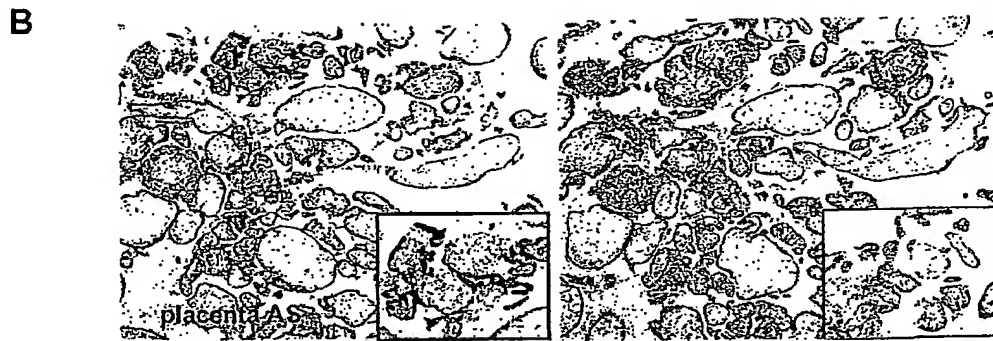
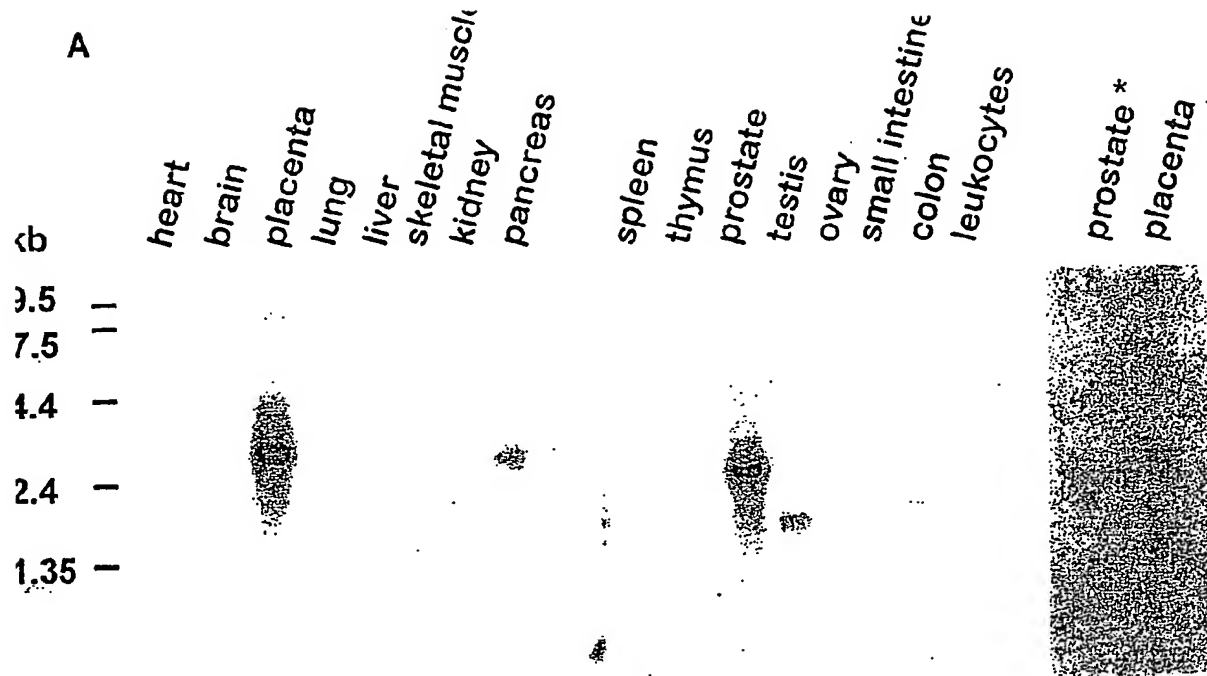


Fig. 6

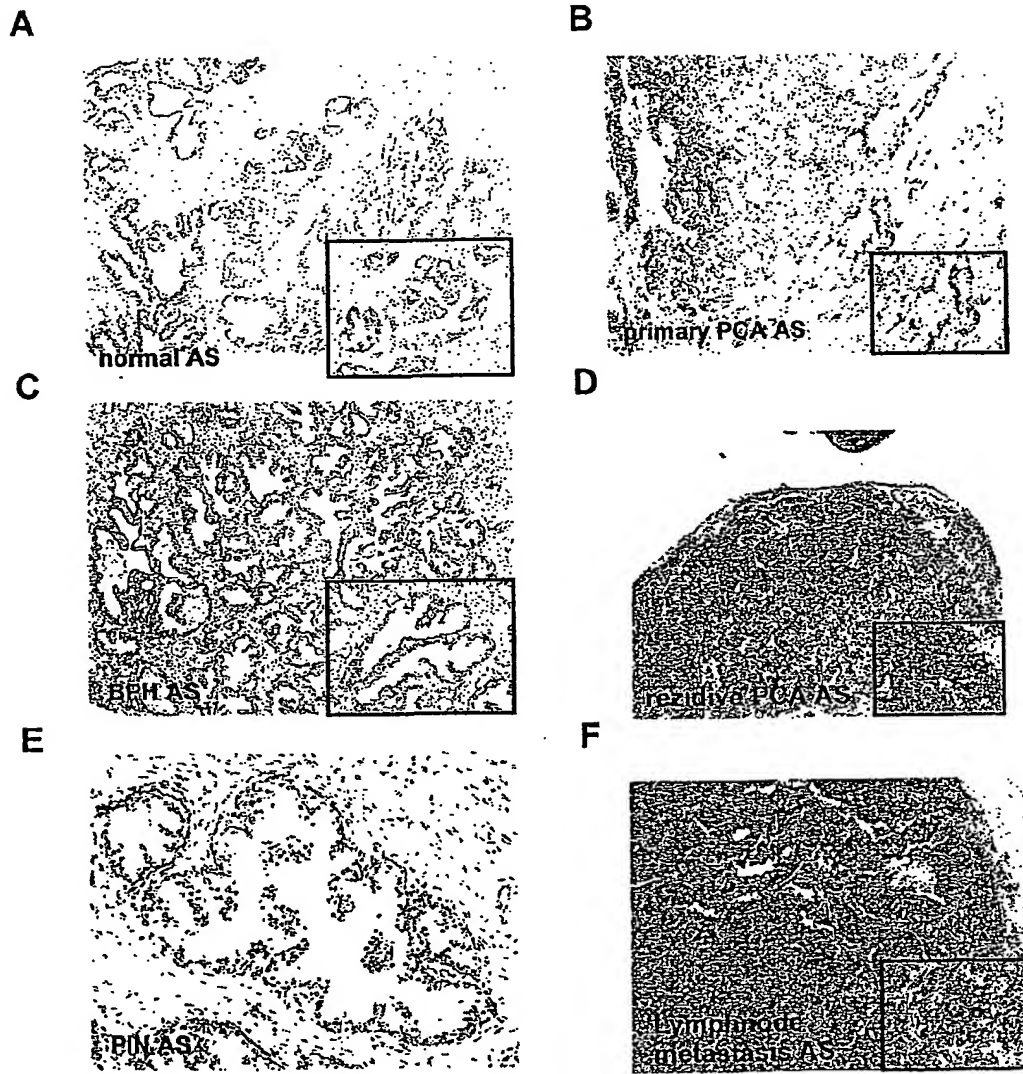


Fig. 7

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      10              30              50
GCCAAGTGTAACTCACTACAGCCCTCTCCAACTGGCTGGGGCTGCTGGGAGACTCCCA
      70              90             110
AGGAACCTCGTCAGGAAGGCAGGAGACAGGAGACGGGACCTCTACAGGGAGACGGTGGGCC
      130             150             170
GGCCCTTGGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGGTCTGGCCTCGGCC
      190             210             230
TCAGGCCCCCAAGGAGCCGGCCCTACACCCCATGGGTTTGTCACTGCCAAGGAGAAAGG
                                M G L S L P K E K G
      250             270             290
GCTAATTCTCTGCCTATGGAGCAAGTTCTGCAGATGGTTCCAGAGACGGGAGTCTTGGGC
      310             330             350
CCAGAGCCGAGATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGGAGTCTCTCTCTCT
      370             390             410
TCTAGCTGCCAAAGATAATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTG
      430             450             470
L A A K D N D V Q A L N K L L K Y E D C
CAAGGTGCACCAGAGAGGAGCCATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGA
      490             510             530
K V H Q R G A M G E T A L H I A A L Y D
CAACCTGGAGGCGCCATGGTGTGATGGAGGCTGCCCCGGAGCTGGTCTTTGAGCCCAT
      550             570             590
N L E A A M V L M E A A P E L V F E P M
GACATCTGAGCTCTATGAGGGTGCAGCTGCAGTGCACATCGCTGTTGTGAACCGAACAT
      610             630             650
T S E L Y E G Q T A L H I A V V N Q N M
GAACCTGGTGCAGCCCTGCTTGCCCGCAGGGCCAGTGTCTCTGCCAGAGCCACAGGCAC
      670             690             710
N L V R A L L A R R A S V S A R A T G T
TGCCCTTCGCGCTAGTCCCCGCAACCTCATCTACTTTGGGGAGCACCCCTTTGTCCTTTGC
      730             750             770
A F R R S P R N L I Y F G E H P L S F A
TGCTGTGTGAACAGTGAAGAGATCGTGGGCTGCTCATTGAGCATGGAGCTGACATCCG
      790             810             830
A C V N S E E I V R L L I E H G A D I R
GGCCAGGACTCCCTGGGAAACACAGTGTACACATCCTCATCCTCCAGCCCAACAAAC
      850             870             890
A Q D S L G N T V L H I L I L Q P N K T
CTTGGCTGCCAGATGTACAACCTGTGTGCTGCTCCTACGACAGACATGGGGACCACCTGCA
      910             930             950
F A C Q M Y N L L L S Y D R H G D H L Q
GCCCTGGACCTCGTGGCCAATCACCAGGGTCTCACCCCTTTCAAGCTGGCTGGAGTGA
      970             990             1010
P L D L V P N H Q G L T P F K L A G V E
GGGTAACACTGTGATGTTTTCAGCACCTGATGCAGAGCGGAAGCACACCCAGTGGACGTA
      1030             1050             1070
G N T V M F Q H L M Q K R K H T Q W T Y
TGGACCACTGACCTCGACTCTCTATGACCTCACAGAGATCGACTCCTCAGGGGATGAGCA
      1090             1110             1130
G P L T S T L Y D L T E I D S S G D E Q
GTCCCTGCTGGAACTTATCATCACCACCAAGAAGCGGGAGGCTCGCCAGATCCTGGACCA
      1150             1170             1190
S L L E L I I T T K K R E A R Q I L D Q
GACGCGGGTGAAGGAGCTGGTGAAGCTCAAGTGAAGCGGTACGGGCGGGCCGTACTTCTG
      1210             1230             1250
T P V K E L V S L K W K R Y G R P Y F C
CATGCTGGGTGCCATATATCTGCTGTACATCATCTGCTTACCAGTGTGCTGCATCTACCG
      1270             1290             1310
M L G A I Y L L Y I I C F T M C C I Y R

```

Fig. 7 / continuation 1

CCCCCTCAAGCCAGGACCAATAACCGCACAGCCCCCGGACACACCTCTTACAGCA
P L K P R T N N R T S P R D N T L L Q Q
1330 1350 1370
GAAGCTACTTCAGGAAGCCTACGTGACCCCTAAGGACGATATCCGGCTGGTCGGGGAGCT
K L L Q E A Y V T P K D D I R L V G E L
1390 1410 1430
GGTGACTGTCAATGGGGCTATCATCATCTGCTGCTAGAGGTTCCAGACATCTTCAGAAT
V T V I G A I I I L L V E V P D I F R M
1450 1470 1490
GGGGGTCACTCGCTTCTTTGGACAGACCATCTTGGGGGCCCATTCATGTCTCATCAT
G V T R F F G Q T I L G G P F H V L I I
1510 1530 1550
CACCTATGCCTTCATGGTGCTGGTGACCATGGTGATGCGGCTCATCAGTGCCAGCGGGGA
T Y A F M V L V T M V N R L I S A S G E
1570 1590 1610
GGTGGTACCATGTCTTTGCACTCGTGTGGGCTGGTGCAACGTATGTACTTCGCCCG
V V P M S F A L V L G W C N V M Y F A R
1630 1650 1670
AGGATTCCAGATGCTAGGCCCTTCACCATCATGATTGAGAAGATGATTTTGGGACCT
G F Q M L G P F T I M I Q K M I F G D L
1690 1710 1730
GATGCGATTCTGCTGGCTGATGGCTGTGGTCATCTTGGGCTTTGCTTCAGCCTTCTATAT
M R F C W L M A V V I L G F A S A F Y I
1750 1770 1790
CATCTTCAGACAGAGGACCCCGAGGAGCTAGGCCACTTCTACGACTACCCCATGGCCCT
I F Q T E D P E E L G H F Y D Y P M A L
1810 1830 1850
GTTCAACCTTCGAGCTGTTCTTACCATCATCGATGGCCAGCCAACTACAACGTGGA
F S T F E L F L T I I D G P A N Y N V D
1870 1890 1910
CCTGCCCTTCATGTACAGCATCACTATGCTGCCTTTGCCATCATGCCACACTGCTCAT
L P F M Y S I T Y A A F A I I A T L L M
1930 1950 1970
GCTCAACCTCCTCATTGCCATGATGGGCGACACTCACTGGGAGTGGCCCATGAGCGGGA
L N L L I A M M G D T H W R V A H E R D
1990 2010 2030
TGAGCTGTGGAGGGCCAGATTGTGGCCACCACGGTGATGCTGGAGCGGAAGCTGCCTCG
E L W R A Q I V A T T V M L E R K L P R
2050 2070 2090
CTGCCTGTGGCCTCGCTCCGGGATCTGCGGACGGGASTATGGCCTGGGGGACCGCTGGTT
C L W P R S G I C G R E Y G L G D R W F
2110 2130 2150
CCTGCGGTGGAAGACAGGCAAGATCTCAACCGGACGGGATCAACGCTACGCACAGGC
L R V E D R Q D L N R Q R I Q R Y A Q A
2170 2190 2210
CTTCCACACCCGGGCTCTGAGGATTTGGACAAAGACTCAGTGGAATACTAGAGCTGGG
F H T R G S E D L D K D S V E K L E L G
2230 2250 2270
CTGTCCCTTCAGCCCCACCTGTCCCTTCTACGCCCTCAGTGTCTCGAAGTACCTCCCG
C P F S P H L S L P T P S V S R S T S R
2290 2310 2330
CAGCAGTGCCAATTGGGAAAGGCTTCGGCAAGGACCCCTGAGGAGAGACCTGCGTGGGAT
S S A N W E R L R Q G T L R R D L R G I
2350 2370 2390
AATCAACAGGGGTCTGGAGGACGGGAGAGCTGGGAATATCAGATCTGACTGCGTGTCT
I N R G L E D G E S W E Y Q I
2410 2430 2450
CACTTCGCTTCCCTGGAACCTTGCTCTCATTTTCTGGGTGCATCAAACAAAAACAAACCA
2470 2490 2510
AACACCCAGAGGTCTCATCTCCAGGCCCCAGGGAGAAAGAGGAGTAGCATGAACGCCAA
2530 2550 2570
GGAATGTACGTTGAGAATCACTGCTCCAGGCTGCATTACTCCTTCAGCTCTGGGGCAGA

Fig. 7 / continuation 2

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2590      2610      2630
GGAAGCCAGCCCAAGCACGGGGCTGGCAGGGCGTGAGGAAGTCTCCTGTGGCCTGCTCA
2650      2670      2690
TCACCCCTCCGACAGGAGCACTGCATGTCAGAGCACTTTAAAAACAGGCCAGCCTGCTTG
2710      2730      2750
GGCCCTCGGTCTCCACCCAGGGTCATAAGTGGGAGAGAGCCCTTCCCAGGGCACCCAG
2770      2790      2810
GCAGGTGCAGGGAAGTGCAGAGCTTGTGGAAGCGTGTGAGTGAGGGAGACAGGAACGGC
2830      2850      2870
TCTGGGGTGGGAAGTGGGGCTAGGTCTTGCCAATCCATCTTCAATAAAGTCGTTTTTCG
2890      2910
GATCCCTAAAAAAAAAAAAAAAAAAAAAAAAA

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MGLSLPKEKGLILCLWSKFCRWFORRESWAQSRDEQNLLQOKRIWESPLLLAAKDNDVQALNKLKYEDCKVHQRGAMGETALHIA
ALYDNLEAMVIMEAAPELVFEPMTSELYEGQTALHIAVNVQNMNLVRALLARRASVSARATGTAFFRRSPRNLIYFGEHPLSFAAC
VNSEEIVRLIEHGADIRAQDSLGNLTVLHILILQPNKTFACQMYNLLSYDRHDLQPLDLVPHQGLTPFKLAGVEGNTVMFQH
LMQKRKHTQWTYGPLTSTLYDLTEIDSSGDEQSLELIITTKREARQILDQTPVKELVSLKWKRYGRPYFCMLGAIYLLYIICFT
MCCIYRPLKPRTNNTSPRDNLLQOKLLQEAYVTPKDDIRLVGELVTVIGAIILLVEVPDIFRMGVTRFFGQTIILGGPFHVLI
TYAFMVLVTVMRLISASGEVVPMSFALVLGWCNVMYFARGFQMLGPFTIMIQLMIFGDLNRFCLMAVVILGFASAFYIIFQTED
PEELGHFYDYPMALFSTFELFLTIIIDGPANYNVDLPFMYSITYAAFAIIATLLMLNLLIAMNGDTHWRVAHERDELWRAQIVATTV
MLERKLPRCLWPRSGICGREYGLGDRWFLRVEDRQDLNRQRIQRYAQAFHTRGSEDLKDSDVEKLELGCFFSPHLSLPTPSVSRST
SRSSANWERLRQGTLLRRDLRGIINRGLEDGESWEYQI

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Figure 8:

A) ATGGGTTTGTCACTGCCCAAGGAGAAAGGGCTAATTCTCT
M G L S L P K E K G L I L C
250 270 290
GCCTATGGAGCAAGTTCTGCAGATGGTTCAGAGACGGGAGTCTGGGCCAGAGCCGAG
L W S K F C R W F Q R R E S W A Q S R D
310 330 350
ATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGGAGTCTCCTCTCCTTCTAGCTGCCA
E Q N L L Q Q K R I W E S P L L L A A K
370 390 410
AAGATAATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTGCAAGGTGCACC
D N D V Q A L N K L L K Y E D C K V H Q
430 450 470
AGAGAGGAGCCATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACAACCTGGAGG
R G A M G E T A L H I A A L Y D N L E A
490 510 530
CCGCCATGGTGCTGATGGAGGCTGCCCGGAGCTGGTCTTTGAGCCCATGACATCTGAGC
A M V L M E A A P E L V F E P M T S E L
550 570 590
TCTATGAGGGTCAGACTGCACATCGCTGTTGTGAACCAGAACATGAACCTGGTGC
Y E G Q T A L H I A V V N Q N M N L V R
610 630 650
GAGCCCTGCTTGCCCGCAGGGCCAGTGTCTCTGCCAGAGCCACAGGCACTGCCTTCCGCC
A L L A R R A S V S A R A T G T A F R R
670 690 710
GTAGTCCCTGCAACCTCATCTACTTTGGGGAGCAOCCCTTTGTCTTTGCTGCCTGTGTGA
S P C N L I Y F G E H P L S F A A C V N

Fig. 8 / contin 11

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      730              750              770
ACAGTGAGGAGATCGTGC GGCTGCTCATTGAGCATGGAGCTGACATCCGGGCCCAGGACT
  S E E I V R L L I E H G A D I R A Q D S
      790              810              830
CCCTGGGAACACAGTGTACACATCCTCATCCTCCAGCCCAACAAAACCTTTGCCTGCC
  L G N T V L H I L I L Q P N K T F A C Q
      850              870              890
AGATGTACAACCTGTGCTGTCTACGACAGACATGGGGACCACCTGCAGCCCTGGACC
  M Y N L L L S Y D R H G D H L Q P L D L
      910              930              950
TCGTGCCCAATCACCAGGGTCTCACCCTTTCAAGCTGGCTGGAGTGGAGGGTAACACTG
  V P N H Q G L T P F K L A G V E G N T V
      970              990              1010
TGATGTTTCAGCACCTGATGCAGAAGCGGAAGCACACCCAGTGGACGTATGGACCACTGA
  M F Q H L M Q K R K H T Q W T Y G P L T
      1030              1050              1070
CCTCGACTCTCTATGACCTCACAGAGATCGACTCCTCAGGGGATGAGCAGTCCCTGCTGG
  S T L Y D L T E I D S S G D E Q S L L E
      1090              1110              1130
AACTTATCATCACCACCAAGAAGCGGGAGGCTCGCCAGATCCTGGACCAGACGCCGGTGA
  L I I T T K K R E A R Q I L D Q T P V K
      1150              1170              1190
AGGAGCTGCTGAGCCTCAAGTGAAGCGGTACGGGCGGCCGTACTTCTGCATGCTGGGTG
  E L V S L K W K R Y G R P Y F C M L G A
      1210              1230              1250
CCATATATCTGCTGTACATCATCTGCTTCACCATGTGCTGCATCTACCGCCCCCTCAAGC
  I Y L L Y I I C F T N C C I Y R P L K P
      1270              1290              1310
CCAGGACCAATAACCGCAGAGCCCCCGGGACAACACCCCTCTTACAGCAGAAGCTACTTC
  R T N N R T S P R D N T L L Q Q K L L Q
      1330              1350              1370
AGGAAGCCTACATGACCCCTAAGGACGATATCCGGCTGGTGGGGAGCTGGTGACTGTCA
  E A Y M T P K D D I R L V G E L V T V I
      1390              1410              1430
TTGGGGCTATCATCCTGCTGGTAGAGGTTCCAGACATCTTCAGATGGGGGCTCACTC
  G A I I I L L V E V P D I F R M G V T R
      1450              1470              1490
GCTTCTTTGGACAGACATCCTTGGGGGCCCATTCATGTCTCATCATCACCTATGCCT
  F F G Q T I L G G P F H V L I I T Y A F
      1510              1530              1550
TCATGGTGTCTGGTGAACATGGTGTATGCGGCTCATCAGTGCCAGCGGGAGGTGGTACCCA
  M V L V T M V M R L I S A S G E V V P M
      1570              1590              1610
TGTCCTTTGCACTCGTGTGGCTGGTGCAACGTCTGTACTTCGCCCCGAGGATTCCAGA
  S F A L V L G W C N V M Y F A R G F Q M
      1630              1650              1670
TGCTAGGCCCTTCAOCATCATGATTCAGAAGATGATTTTGGCGACCTGATGCGATTCT
  L G P F T I M I Q K M I F G D L M R F C
      1690              1710              1730
GCTGGCTGATGGCTGTGGTCACTCCTGGGCTTTGCTTCAGCCTTCTATATCATCTTCCAGA
  W L M A V V I L G F A S A F Y I I F Q T
      1750              1770              1790
CAGAGGACCCCGAGGAGCTAGGCCACTTCTACGACTACCCCATGGCCCTGTTTCAGCACCT
  E D P E E L G H F Y D Y P M A L F S T F
      1810              1830              1850
TCGAGCTGTTTCTTACCATCATCGATGGCCAGCCAACTACAACGTGGACCTGCCCTTCA
  E L F L T I I D G P A N Y N V D L P F M
      1870              1890              1910
TGTCAGCATCACCTATGCTGCTTGGCATCATCGCCACACTGCTCATGCTCAACCTCC
  Y S I T Y A A F A I I A T L L M L N L L
      1930              1950              1970
TCATTGCCATGATGGCGGACACTCACTGGCGAGTGGCCCATGAGCGGGATGAGCTGTGGA

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Fig. 8 / conti: on 2

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      I A M M G D T H W R V A H E R D B L W R
      1990                      2010                      2030
      GGGCCAGATTGTGGCCACCACGGTGATGCTGGAGCGGAAGCTGCCTCGCTGCCTGTGGC
      A Q I V A T T V M L E R K L P R C L W P
      2050                      2070                      2090
      CTGCTCCGGGATCTGCGGACGGGAGTATGGCTGGGAGACCGCTGGTTCCTGCGGGTGG
      R S G I C G R E Y G L G D R W F L R V E
      2110                      2130                      2150
      AAGACAGGCAAGATCTCAACCGGCAGCGGATCCAACGCTACGCACAGGCCTTCCACACCC
      D R Q D L N R Q R I Q R Y A Q A F H T R
      2170                      2190                      2210
      GGGGCTCTGAGGATTGGACAAAGACTCAGTGGAAAACTAGAGCTGGGCTGTCCCTTCA
      G S E D L D K D S V E K L E L G C P F S
      2230                      2250                      2270
      GCGCCCACTGTCCCTTCTATGCCCTCAGTGTCTCGAAGTACCTCCCGCAGCAGTGCCA
      P H L S L P M P S V S R S T S R S S A N
      2290                      2310                      2330
      ATTGGGAAAGGCTTCGGCAAGGGACCTGAGGAGAGACCTGCGTGGGATAATCAACAGGG
      W E R L R Q G T L R R D L R G I I N R G
      2350                      2370                      2390
      GTCTGGAGGACGGGAGAGCTGGGAATATCAGATCTGA
      L E D G E S W E Y Q I *

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MGLSLPKEKGLILCLWSKFCRWFQRRESWAQSRDEQNLIQOKRIWESPLLLAAKDNVDQALNKLKYE DCKVHQRGAMGETALHIA
 ALYDNLEAANVLM EAPELVFEPM TSELYEGQ TALHIAV VNONMNLVRALLARRASVSARATGTAFRRSPCNLIYFGEHPLSFAAC
 VNSEEIVRLLEH GADIRAQDSLGN TVLHILILQPNKT FACQMYNLLSYDRHGDHLQPLDLVFNHQGLT PFKLAGVEGNTVMFOH
 LMQKRKHTQW TYGLPTSTLYDLTEIDSSGDEQSLELELIITTKREARQILDQTPVKELVSLKWKRYGRPYFCMLGAIYLLYIICFT
 MCCIYRELKPRTNRTSPRDN TLLQOKLLQEA YMTPEKDDIRLVGELVTVIGAJIILLVEVPDIFRMGVTRFEGQTLILGPFPHVLII
 TYAFMVLVTNMRLISASGEVVMSFALVLGWCNVMYFARGFQMLGPFTIMI QKMIFGDLMREFCWLM AVVILGFASAFYIIFQTED
 PEELGHFYDYPMALFSTFELFLTIIIDGPANYNDLFFMYSITYAFAIIATLLMLNLLIAMMGDTHWRVAHERDELWRAQIVATTV
 MLERKLPRCLWPRSGICGREYGLGDRWFLRVEDRQDLNRQRIQRYAQAFHTRGSEDLDKDSVEKLELGCPFSPHLSLEPNPSVSRST
 SRSSANWERLRQGLRRDLRGIINRGLEDGESWEYQI

B)

CAAACTCACAGCCCTCTCAAAC TGGCTGGGGCTGCTGGGAGACTCCCAAGGAAC TCGTCAGGAAGGCAGGAGACAGGAGACGGGA
 CCTCTACAGGAGAGCGGTGGGCCGGCCCTTGGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGGTCTGGCCTCGGCCCTCA
 GGCCCCCAAGGAGCGGCCCTACACCCCATGGGTTGTCACTGCCCAAGGAGAAAGGGCTAATTCTCTGCCTATGGAGCAAGTTCT
 GCAGATGGTTCAGAGACGGGAGTCTGGGCCAGAGCCGAGATGAGCAGAACCTGCTGCAGCAGAAAGAGATCTGGGAGTCTCCT
 CTCCTTCTAGCTGCCAAGATAATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTCAGAGTGCACACAGAGAGAGC
 CATGGGGGAACAGCGCTACACATAGCAGCCCTCTATGACAACCTGGAGGCCGCCATGCTGCTGATGGAGGCTGCCCGGAGCTGG
 TCTTTGAGCCCATGACATCTGAGCTCTATGAGGGTCAGACTGCAGTGCACATCGCTGTGTGAACCAAGACATGAACCTGGTGCAG
 GCCCTGCTTGCCCGCAGGGCCAGTGTCTTGCCAGAGCCACAGGCACTGCCTTCGCGCCTAGTCCCGCAACCTCATCTACTTTGG
 GGAGCACCTTTGCTCTTGTGCTGCTGTGAACAGTGAGGAGATCGTGGGCTGCTCATTGAGCATGGAGCTGACATCGGGGCC
 AGGACTGCCTGGCCCAACAAACCTTTGCCCTGCCAGATGTACACCTGTTGCTGTCTACGACAGACATGGGGACCACTGCAGCC
 CCTGGACCTCGTGCCCAATCACCAGGGTCTCACCCCTTTCAAGCTGGCTGGAGTGGAGGTAACACTGTGATGTTTCAGCACCTGA
 TGCAGAAGCGGAAGCACACCCAGTGGACGTATGGACCACTGACCTCGACTCTCTATGACCTCACAGAGATGACACTCCTCAGGGGAT
 GAGCAGTCCCTGCTGGAACCTTATCATCACCACCAAGAACGGGAGGCTCGCCAGATCCTGGACAGACGCGGGTGAAGGAGCTGGT
 GAGCCTCAAGTGAAGCGGTACGGGCGGCCGTACTTCTGCACTGCTGGGTGCCATATATCTGCTGTACATCATCTGCTTCAACATGT
 GCTGCACTACCGCCCCCTCAAGCCAGGACCAATAACCGCACAGCCCCGGGACAAACCCCTTTACAGCAGAGCTACTTCAG
 GAAGCCTACGTGACCCCTAAGGACGATATCCGGCTGGTGGGGAGCTGGTGACTGTCAATTGGGGCTATCATCATCTGCTGGTAGA
 GGTTCAGACATCTTCAGAAATGGGGTCACTCGCTTCTTTGGACAGACCATCCTTGGGGGCCCATTCATGTCCTCATCATCACT
 ATGCCTTCATGGTGTGGTGACCATGGTGATGCGGCTCATATGATTTTGGCGACCTGATGCGATTCTGCTGGCTGATGGCTGTGG
 TCATCTGGGCTTTGCTTCAGCCTTCTATATCATCTTCAGACAGAGGACCCGAGGAGCTAGGCCACTTCTACGACTACCCCATG
 GCCCTGTTACGACCTTCGAGCTGGTCTTACCATCATCGATGGGCCAGCCAACTACAACGTGGAACCTGCCCTTCATGTACAGCAT
 CACCTATGCTGCCTTGGCATCATCGCCACACTGCTCATGCTCAACCTCCTCATTGCCATGATGGGGACACTCACTGGGAGTGG
 CCCATGAGCGGGATGAGCTGTGGAGGGCCAGATTGTGGCCACCAOGGTGATGCTGGAGCGGAAGCTGCCTCGCTGCCTGTGGCT
 CGCTCCGGGATCTGCGGACGGGAGTATGGCTGGGGGACCGCTGGTTCCTGCGGGTGGAGACAGGCAAGATCTCAACCGGCAGCG

Fig. 8 / continuation 3

GATCCAACGCTACGCACAGGCCTTCCACACCCGGGGCTCTGAGGATTTGGACAAAGACTCAGTGGAAAACTAGAGCTGGGCTGTC
 CCTTCAGCCCCACCTGTCCCTTCTACGCCCTCAGTGTCTCGAGTACCTCCCGCAGCAGTGCCAATTGGGAAAGGCTTCGGCAA
 GGGACCTTGAGGAGAGACCTGCGTGGGATAATCAACAGGGGTCTGGAGGACGGGGAGAGCTGGGAATATCAGATCTGACTGCGTGT
 TCTCACTTCGCTTCCTGGAACCTTGTCTCATTTTCTGGGTGCATCAAAACAAAACAAAACCAACACCCAGAGGTCTCATCTCCC
 AGGCCCCAGGGAGAAAGAGGAGTAGCATGAACGCCAAGGAATGTACGTTGAGAATCACTGCTCCAGGCCCTGCATTACTOCTTCAGC
 TCTGGGGCAGAGGAAGCCAGCCCAAGCACGGGGCTGGCAGGGCTGAGGAACCTCTCTGTGGCTGCTCATCACCTTCCGACAG
 GAGCACTGCATGTCAGAGCACTTTAAAAACAGGCCAGCCTGCTTGGGCCCTCGGTCTCCACCCAGGGTCATAAGTGGGGAGAGAG
 CCCTTCCAGGGCACCAGGCAGGTGCAGGAAGTGCAGAGCTTGTGGAAGCGTGTGAGTGAGGGAGACAGGAACGGCTCTGGGG
 GTGGGAAGTGGGGCTAGGTCTTGCCAACTCCATCTTCAATAAAGTCGTTTTCGGATCCCTAAAAA

c.)

CAAACTCACAGCCCTCTCCAACTGGCTGGGGCTGCTGGGAGACTCCCAAGGAACCTGTCAGGAAGGCAGGAGACAGGAGACGGGA
 CCTCTACAGGGAGACGGTGGGCCGGCCCTTGGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGGTCTGGCCTCGGCCCTCA
 GGCCCCAAGGAGCGGCCCTACACCCCATGGGTTTGTCACTGCCAAGGAGAAAGGGCTAATTCTCTGCCATATGGAGCAAGTTCT
 GCAGATGGTTCCAGAGACGGGAGTCTTGGGCCCAGAGCCGAGATGAGCAGAACTGCTGCAGCAGAAAGGATCTGGGAGTCTCCT
 CTCTTCTAGCTGCCAAGATAATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTGCAAGGTGCACCAGAGAGGAGC
 CATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACAACTGGAGGCCCATGCTGCTGATGGAGGCTGCCCGGAGCTGG
 TCTTTGAGCCCATGACATCTGAGCTCTATGAGGTCTGACTGCCCATCACTTGAAACGCTGCCCTTGAATGCCAGGCCCTAGAG
 AAGAGGAAGAGATGGGCAGCAGCTGGATCCCTGGGAATCTGAACACCCGAGAGCTCCCTGTTCTCCATCCCAGGCTACCCCTGA
 GGGAAAGAGACTGGGTGCATATGGGAGGGACCCCTGCAGGATCCTGGGACAGACCCGCTGACTGACAGTGTCTCTGGGCCAGG
 TCAGACTGCACTGCACATCGCTGTGTGAACCAAGAACATGAACCTGGTGCAGGCCCTGCTTGCCCGCAGGGCCAGTGTCTCTGCCA
 GAGCCACAGGCCTGCTTCCGCGTAGTCCCTGCAACCTCATCTACTTTGGGGAGCACCCCTTGTCTTGTGCTGTGTGAAC
 AGTGAGGAGATCGTGGGCTGCTCATTTGAGCATGGAGCTGACATCGGGGCCAGGACTCCCTGGCCCAACAAAACCTTTGCTGCC
 AGATGTACAACTGTTGCTGTCTACGACAGACATGGGGACCACTGCAGCCCTGGACCTCGTGCCCAATCACCAGGGTCTCACC
 CCTTTCAAGCTGGCTGGAGTGGAGGGTAACACTGTGATGTTTTCAGCACCTGATGCAGAAAGCGGAAGCACACCCAGTGGACGTATGG
 ACCACTGACCTCGACTCTATGACCTCACAGAGATCGACTCTCAGGGGATGAGCAGTCCCTGCTGGAACCTATCATCACCAACA
 AGAAGCGGGAGGCTCGCCAGATCTGGACCAAGACCGCGTGAAGGAGCTGGTGAAGCTCAAGTGAAGCGGTACGGCGGCCGTAC
 TTCTGCATGCTGGGTGCCATATATCTGCTGTACATCATCTGCTTCAACATGTGCTGCATCTACCGCCCCCTCAAGCCAGGAACAA
 TAACCCGACGAGCCCCCGGACAAACCCCTCTTACAGCAGAAGCTACTTCAGGAAGCCTACATGACCCCTAAGGACGATATCGGC
 TGGTCCGGGAGCTGGTGAAGTGTCTTGGGGCTATCATCATCTGCTGGTAGAGGTTCCAGACATCTTCAGAAATGGGGTCACTGCG
 TTCTTTGGACAGACCATCTTGGGGGCCATTCATGTCTCATCATCACCTATGCTTTCATGGTGTGGTGACCATGGTGTATGG
 GCTCATCAGTGCCAGCGGGAGGTGGTACCCATGTCTTTGCACTGCTGCTGGGCTGGTGCAACGTATGTAATTCGCCCGAGGAT
 TCCAGATGCTAGGCCCCCTTACCATCATGATTGAGAAGATGATTTTGGCGACCTGATGCGATTCTGCTGGCTGATGGCTGTGGTC
 ATCTGGGCTTTGCTTAGACAGAGGACCCGAGGAGCTAGGCCACTTCTACGACTACCCCATGGCCCTGTTGAGCACCTTCAGACT
 GGTCTTACCATCATGATGGCCAGCCAACTACACGTTGAGCTGCTGCTTTCATGTACAGCATCACCTATGCTGCTTTGCCATCA
 TCGCCACACTGCTCATGCTCAACCTCTCTATTGCCATGATGGGCGACACTCACTGGCGAGTGGCCCATGAGCGGGATGAGCTGTGG
 AGGGCCAGATTGTGGCCACCAAGGTGATGCTGGAGCGGAAGCTGCTGCTGCTGTGGCTGCTGCTGGGATCTGGGACGGGA
 GTATGGCTTGGGAGACCGCTGGTTCCTGCGGGTGGAGACAGCAAGATCTCAACCGGCAGCGGATCCAAACCTACGCACAGGCCT
 TCCACACCCGGGGCTCTGAGGATTGGACAAAGACTCAGTGGAAAACTAGAGCTGGGCTGTCCCTTCAGCCCCACCTGTCCCTT
 CCTATGCCCTCAGTGTCTCGAAGTACCTCCGCGCAGCAGTCCAAATTGGGAAAGGCTTCGGCAAGGGACCTGAGGAGAGACCTGCG
 TGGGATAATCAACAGGGGTCTGGAGGACGGGGAGAGCTGGGAATATCAGATCTGACTGCGTGTCTCACTTCCGCTTCTGGAACCT
 GCTCTCATTTCTGGGTGCATCAAAACAAAACAAAACCAACCCAGAGGTCTCATCTCCAGGCCCGAGGAGAAAGAGGAGT
 AGCATGAACGCCCAAGGAATGTACGTTGAGAATCACTGCTCCAGGCCCTGCATTACTCTTCAGCTCTGGGGCAGAGGAAGCCAGGCC
 CAAGCACGGGGCTGGCAGGGCGTGAGGAACCTCTCTGTGGCCTGCTCATCACCTTCCGACAGGAGCATGCATGTGAGAGCACTT
 TAAAAACAGGCCAGCCTGCTTGGGCCCTCGGTCTCCACCCAGGGTCATAAGTGGGGAGAGAGCCCTTCCAGGGCACCCAGGCAG
 GTGCAGGGAAGTGCAGAGCTTGTGGAAGCGTGTGAGTGAGGAGACAGGAACGGCTCTGGGGGTGGGAAGTGGGGCTAGGTCTTGG
 CCAACTCCATCTTCAATAAAGTCGTTTTCGGATCCCTAAAAA

d.)

CAAACTCACAGCCCTCTCCAACTGGCTGGGGCTGCTGGGAGACTCCCAAGGAACCTGTCAGGAAGGCAGGAGACAGGAGACGGGA
 CCTCTACAGGGAGACGGTGGGCCGGCCCTTGGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGGTCTGGCCTCGGCCCTCA
 GGCCCCAAGGAGCGGCCCTACACCCCATGGGTTTGTCACTGCCAAGGAGAAAGGGCTAATTCTCTGCCATATGGAGCAAGTTCT
 GCAGATGGTTCCAGAGACGGGAGTCTTGGGCCCAGAGCCGAGATGAGCAGAACTGCTGCAGCAGAAAGGATCTGGGAGTCTCCT
 CTCTTCTAGCTGCCAAGATAATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGCATGCAAGGTGCACCAGAGAGGAGC
 CATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACAACTGGAGGCCCATGGTCTGATGGAGGCTGCCCGGAGCTGG
 TCTTTGAGCCCATGACATCTGAGCTCTATGAGGGTCAGACTGCACTGCACATCGCTGTGTGAACCAAGATGAACCTGGTGGCA
 GGCCCTGCTTCCCGCAGGGCCAGTGTCTCTGCCAGGCCACAGGCACTGCCTTCCGCGTAGTCCCGCAACCTCATCTACTTTGG

AATACACAGCTGTTACACATCCTCATCCTCCAGCCCAACAAAACCTTTGCCTGCCAGATGTACAACTGTTGCTGTCTTACGACAGAC
 ATGGGACCACCTGACGCCCCCTGGACCTCGTGCCCAATCACCAGGCTCTCACCCTTTCAAGCTGGCTGGAGTGGAGGGTAACACT
 GTGATGTTTTGACACCTGATGCAAGAAGCGGAAGCACACCCAGTGGACGTATGGACCATTGACCTCGACTCTCTATGACCTCACAGA
 GATCGACTCCTCAGGGGATGAGCAGTCCCTGCTGGAATTTATCATCACCACCAAGAAGCGGGAGGCTCGGCAGATCCTGGACAGA
 CGCCGGTGGAAGGAGCTGGTGACCTCAAGTGAAGCGGTACGGCGCGCTACTTCTGCATCTGCGGTGCCATATATCTGCTGTAT
 ATCATCTGCTTACCAGTGTGCTGCATCTACCGCCCCCTCAAGCGCCAGACCAATTAACCGCACAAAGCCCCGGGACAACACCTCTT
 ACAGCAGAAGCTACTTTCAGGAAGCCTACGTGACCCCTAAGGACGATATCCGGCTGGTGGGGAGCTGGTGACTGTCATTGGGGCTA
 TCATCATCTGCTGGTAGAGGTTCCAGACATCTTCAGAAATGGGGTCACTCGCTTCTTTGGACAGACCATCCTTGGGGGCCATT
 CATGTCCTCATCATCACCTATGCTTTCATGTGCTGGTGACCATGGTGTGCGGCTCATCAGTGCCAGCGGGAGGTTGGTACCCAT
 GTCCTTTGCACTCGTGCTGGGCTGGTGCAACGTGATCTACTTCGCCCCAGGATTCAGATGCTAGGCCCCCTCACCATTCAATGATC
 AGAAGATGATTTTTGGCGCAGCTGATGCGATCTGCTGGCTGATGGCTGTGGTCACTCGGCTTTGCTTCAGCCTTCTATATCATC
 TTTCCAGACAGGAGCCCGAGGAGCTAGGCCACTTCTACGATACCCCATGCCCTGTTTTCAGACATCTCGAGCTGGTCTTACCAT
 CATCGATGGCCGCGCACTACACAGCTGGACCTGCCCTTCATGTACAGCATCACCTATGCTGCTTTGGCCATCATCGCCCACTGC
 TCATGCTCAACCTCCTCATTGCCATGATGGGCGACACTCACTGGCGAGTGGCCCATGAGCGGGATGAGCTGTGGAGGGCCAGATT
 GTGGCCACCACGGTGATGCTGGAGCGGAAGCTGCCCTCGCTGCTGTGGCCCTCGCTCCGGGATCTCGCGACGGGAGTATGGCTGGG
 GGACCCGCTGGTTCTGCGGGTGAAGACAGGCAAGATCTCAACCCGCGAGCGGATCCAACGCTACGCACAGGCCTTCCACACCGGG
 GCTCTGAGGATTTGGACAAAAGACTCAGTGGAAAACTAGAGCTGGGCTGCTCCTTCAGCCCCACAGTGCCTTCTCAACGCCCTCA
 GTGCTCGAAGTACCTCCCGACAGCATGCCAATTGGGAAGGTTTCGGCAAGGACCTCGAGGAGACCTGAGGAGACAGCTCGTGGGTAATCA
 CAGGGGCTCTGGAGAGCGGGAGAGCTGGGAATTAGATCTGACTGCGTGTTCTCACTTCGCTTCTGGAACCTGCTCTCATTTTC
 CTGGGTGCATCAAAACAAAACAAAACCAACACCCAGAGGTCTCATCTCCAGGCCCCAGGGAGAAAGAGGAGTAGCATGAACGCC
 AAGGAATGTACGTTGAGAATCACTGCTCCAGGCCTGCATTAATCTCTCAGCTCTGGGGCAGAGGAAGCCCAAGCCCAAGCACGGGG
 TGGCAGGGCTGAGGAATCTCTCTGTGGCCTGCTCATCAACCTTCCGACAGGAGCACTGCATGTCTCAGAGCACTTTAAAAACAGGCC
 AGCCTGCTTGGGCCCTCGGTCTCCACCCAGGGTCTAAGTGGGGGAGAGGCCCTTCCAGGGCACCCAGGCAGGTGACAGGAAGT
 GCAGAGCTTGTGGAAGCGTGTGATGTAGGAGACAGGAACGGCTCTGGGGGTGGGAAGTGGGGCTGCTGCAACTTCCATCT
 TCAATAAAGTCTGTTTTGCGATCCTTAAAAAATAAAAAAAAAAAAAAAAAA

CACACATGGGGCCTCCCAGGAGTGCCCAGGACCTCGTGCTGTTGGCCCTCTGAATCTATCGTCTCCAATCCGCTGTCCCACAGAAGC
CATATAACCCACCTCTCTGTAATAGCCAGGAGCCATGGGGGAAACAGCGCTACACATAGACGCTCCCTCTATGACAAACCTGGAGGCGG
CCATGGTGCTGATGGAGGCTGCCCCGGAGCTGGTCTTTGAGCCCATGACATCTGAGCTCTATGGAGGGTGAGGGCCACCGGCTCTG
GGGTGAAGAGCAGGAGTGACGTGTTGGTATTCAGTCAGTCTGTCATGATGATAATTTGGGAAGACACAGGGGATCTGTAGCCT
CCTACTCTTTTTSTCTPCTCTGTCTCCCTTCOGTGTGAGTCCCTGACTGCCCATCACTTGAACGCTTGCCCCCTGAAATGCCAGGG
GCCTAGAGAAGAGGAAGAGATGGGCAGCAGCTGGATCCCTGGGAATCCTGAACACCCGAGAGCTCCCTGTTCTCCATCCCAGGCT
ACCCCTGAGGGAAAGAGACTAGGGGTGCATATGGGAGGGGACCCCTGCAAGGATCCTAGGGGACAGACCCGCTGACTGACAGCTGTCT
CTGGGCCAGGTGCAGACTGCATGCACTGCTGTTGTGAACAGGAACATGAACCTGGTGGAGCCCTGCTTGCCCGCAGGGCCAGT
GTCTCTGCCAGAGCCACAGGCATGCCTCCCGCGTAGTCCCTGCAACCTCATCTACTTTGGGGAGCACCCCTTGTCTTTGCTGC
CTGTGTGAACAGTGAGGAGATCGTGGCGGCTGCTCATTGAGCATGAGAGTGACATCCGSGCCAGGACTCCCTGGATGTACAACCTG
TTGCTGTCTACGACAGACATGGGGACCACCTGCAGCCCTGGACCTCGTGCCCAATCACCAGGGTCTCACCCCTTTCAAGCTGGC
TGGAGTGGAGGGTAACACTGTGATGTTTCAGCACCTGATGCAGAAGCGGAAGCACACCCAGTGGAGCTATGGAGCCACTGACCTCGA
CTCTCTATGACCTCACAGAGATCGACTCTCCAGGGGATGAGCAGCTCCCTGTGGAACCTTATCATCACCACAGAAGCGGGAGGCT
CGCCAGATCCTGGACAGACGCGGCTGAAGAGCTGTGAGCCTCAAGTGAAGCGGTACGGCGCGGTACTTCTGCATGCTGGG
TGCCATATATCTGCTGTACATCATCTGCTTACCATGTGCTGCATCTACGCCCCCTACGCCCAGGACCAATAACCGCAOGAGCC
CCCGGGAACACCCCTCTTACAGCAGAAGCTACTTCAGGAAGCCTACATGACCCCTAAGGACGATATCCGCTGGTGGGGAGCTG
GTGACTGTCAATTGGGGCTATCATCATCCTGCTGGTAGAGGTTCCAGACATCTTCAAGATGGGGGTCACTCGCTTCTTTGGACAGAC
CATCCTTGGGGGCCCATTCATGTCTCATCATCACTTATGCCTTCATGTGCTGGTGACCTGGTGTGCTGGGCTCATCATGTGCCA
CGCGGGAGGTGGTACCATTGCTTTGCACTCGCTGGCTGGGTGGTGAACCTCATGTACTTGCGCCAGGAGTTCAGATGCTTAGGC
CCCTTCAACATCATGATTCATGAAAGATGATTTTGGCGACCTGATGCGATTCTGTGTGCTGATGGCTGTGGTCACTCGGGCTTGTG
TTCAGCCTTCTATATCATCTTCCAGACAGAGGACCCCGAGGAGCTAGGCCACTTCAOAGCTACCCCATGGCCCTGTTACGACCT
TCGAGCTGGTCTTACCATCATCGATGGCCAGCCAACTACAACGTGGAACCTGCCCTTCATGTACAGCATCACTATGCTGCCTTT
GCCATCATCGCCCACTGCTCATGCTCAACCTCCTCATTTGCCATGATGGCGACACTCACTGCGGAGTGGCCCATGAGCGGGATGA
GCTGTGGAGGGCCAGATTGTGGCCACCACGGTGATGCTGGAGCGGAAGCTGCTCGTGCCTGTGGCTCGCTCCGGGATCTGG
CAGCGGAGTATGGCTGGGAGACCTGCTGTTCTCGGGGTGAAGACAGGCAGATCTCAACCGGAGCGGATCAACGCTACGCA
GACGGCTTCCACACCCCGGGCTCTGAGGATTGGACAAGAACTCAGTGGAAAACTAGAGCTGGGCTGTCCCTCAGCCCCACCT
GTCCCTTCTATGCCCTCAGTGTCTCGAAGTACCTCCCGCAGCAGTGGCAATTGGGAAGGCTTCGGCAAGGGACCCCTGAGGAGAG
AOCCTGCGTGGGATATCAACAGGGGTCTGGAGGACGGGAGAGCTGGGAATATCAGATCTGACTGCGTGTCTCACTTCGCTTCT
GGAACCTGTCTCATTTTCTGGGTGCATCAAAACAAACAAACACCCAGAGGTCTCATCTCCAGGCCCCAGGGAGAA
GAGGAGTAGCATGAACCGCAAGGAATGACGTGTGAGAATCTCTGCTCCAGGCTGCATTACTCTTCAGCTTCTGGGGCAGGAGAG
CCGACGCCAAGCACGGGCTCGCAGGGGTGAGGAACCTCCTGTGGCCCTGCTCATACCTTCCGACAGGACATGCATGTCAAG
AGCATTTAAAAACAGGCCAGCTGCTGTTGGGCCCTCGGTCTCCACCCAGGGTCATAGTGGGGAGAGAGCCCTTCCAGGGCACC

Fig. 8 / continuation 5

CAGGCAGGTGCAGGGAAGTGCAGAGCTTGTGAAAGCGTGTGAGTGAGGGAGACAGGAACGGCTCTGGGGGTGGGAAGTGGGGCTA
GGTCTTGCCAACTCCATCTTCAATAAAGTCGTTTTCGGATCCTAAAAAAAAAAAAAAAAAAAAAAAAA

Figure 9:

A.

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      10      30      50
CGGGGCCCTGGGCTGCAGGAGGTTGCGGCGGCCGCGGCAGCATGGTGGTGCCGGAGAAGG
                                     M V V P E K E
      70      90      110
AGCAGAGCTGGATCCCAAGATCTTCAAGAAGAAGACCTGCACGACGTTCATAGTTGACT
Q S W I P K I F K K K T C T T F I V D S
      130      150      170
CCACAGATCCGGGAGGGACCTTGTGCCAGTGTGGGCGCCCCCGGACCGCCCAACCCGCGAG
T D P G G T L C Q C G R P R T A H F A V
      190      210      230
TGGCCATGGAGGATGCCTTCGGGGCAGCCGTGGTGACCGTGTGGGACAGCGATGCACACA
A M E D A F G A A V V T V W D S D A H T
      250      270      290
CCACGGAGAAGCCCAACCGATGCCTACGGAGAGCTGGACTTCACGGGGCGCCGCCCAAGC
T E K P T D A Y G E L D F T G A G R K H
      310      330      350
ACAGCAATTTCTCCGGCTCTCTGACCGAACGGATCCAGCTGCAGTTTATAGTCTGGTCA
S N F L R L S D R T D P A A V Y S L V T
      370      390      410
CAOGCAGATGGGGCTTCCGTGCCCCGAACCTGGTGGTGTGAGTGCTGGGGGATCGGGGG
R T W G F R A P N L V V S V L G G S G G
      430      450      470
GCCCCGTCTCCAGACCTGGCTGCAGGACCTGCTGCGTCTGGGGCTGGTGCGGGCTGCCC
P V L Q T W L Q D L L R R G L V R A A Q
      490      510      530
AGAGCAGAGGAGCCTGGATTGTCACTGGGGGTCTGCACACGGGCATCGGCCGGCATGTTG
S T G A W I V T G G L H T G I G R H V G
      550      570      590
GTGTGGCTGTACGGGACCATCAGATGGCCAGCACTGGGGGCACCAAGGTGGTGGCCATGG
V A V R D H Q M A S T G G T K V V A M G
      610      630      650
GTGTGGCCCCCTGGGGTGTGGTCCGGAATAGAGACACCTCATCAACCCCAAGGGCTCGT
V A P W G V V R N R D T L I N P K G S F
      670      690      710
TCCCTGCGAGGTACCGGTGGCGCGGTGACCCGGAGGACGGGGTCCAGTTTCCCCTGGACT
P A R Y R W R G D P E D G V Q F P L D Y
      730      750      770
ACAACTACTCGGCCCTTCTTCTGGTGGACGACGGCACACACGGCTGCCTGGGGGGCGAGA
N Y S A F F L V D D G T H G C L G G E N
      790      810      830
ACCGCTTCCGCTTGGCCCTGGAGTCCTACATCTCACAGCAGAAGACGGGCGTGGGAGGGA
R F R L R L E S Y I S Q Q K T G V G G T
      850      870      890
CTGGAATTGACATCCCTGTCTCTCTCTGATTGATGGTGATGAGAAGATGTTGACGC
G I D I P V L L L L I D G D E K M L T R
      910      930      950
GAATAGAGAAGCCACCCAGGCTCAGCTCCCATGTCTCCTCGTGCTGGCTCAGGGGGAG
I E N A T Q A Q L P C L L V A G S G G A
      970      990      1010
CTGCGGACTGCCTGGCGGAGACCTGGAAGACACTCTGGCCCCAGGGAGTGGGGGAGCCA
A D C L A E T L E D T L A P G S G G A R
      1030      1050      1070
GGCAAGGCGAAGCCGAGATCGAATCAGGCGTTTCTTTCCCAAGGGGACCTTGAGGTCC

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Fig. 9 / continuation 1

Q G E A R D R I R R F F P K G O L E V L
 1090 1110 1130
 TGCAGGCCAGGTGGAGAGGATTATGACCCGGAAGGAGCTCCTGACAGTCTATTCTTCTG
 Q A Q V E R I M T R K E L L T V Y S S E
 1150 1170 1190
 AGGATGGGTCTGAGGAATTCGAGACCATAGTTTGAAGGCCCTTGTGAAGGCCTGTGGGA
 D G S E E F E T I V L K A L V K A C G S
 1210 1230 1250
 GCTCGGAGGCCTCAGCCTACCTGGATGAGCTGCCCTTGGCTGTGGCTTGAACCGCGTGG
 S E A S A Y L D E L R L A V A W N R V D
 1270 1290 1310
 ACATTGCCAGAGTGAAGTCTTTCCGGGGGACATCCAATGGCGCTCCTTCCATCTCGAAG
 I A Q S E L F R G D I Q W R S F H L E A
 1330 1350 1370
 CTTCCCTCATGGACGCGCTGCTGAATGACCGGCTGAGTTCTGCGCTTGCTCATTTCC
 S L M D A L L N D R P E F V R L L I S H
 1390 1410 1430
 ACGCCTCAGCCTGGGCCACTTCCCTGACCCGATGCGCCTGGCCCAACTCTACAGCGCGG
 G L S L G H F L T P N R L A Q L Y S A A
 1450 1470 1490
 CGCCTCCAAGTCTGCTCATCCGCAACCTTTGGACCAAGCGTCCACAGCGCAGGCACCA
 P S N S L I R N L L D Q A S H S A G T K
 1510 1530 1550
 AAGCCCCAGCCCTAAGAGGGGGAGCTGCGGAGCTCCGGCCCCCTGACGTGGGGCATGTGC
 A P A L K G G A A E L R P P D V G H V L
 1570 1590 1610
 TGAGGATGCTGCTGGGGAAGATGTGCGCGCGAGGTACCCCTCCGGGGCGCCTGGGACC
 R M L L G K M C A P R Y P S G G A W D P
 1630 1650 1670
 CTCACCCAGGCCAGGGCTTCGGGGAGAGCATGTATCTGCTCTCGGACAAAGGCCACCTGCG
 H P G Q G F G E S M Y L L S D K A T S P
 1690 1710 1730
 CGCTCTCGCTGGATGCTGGCCTCGGGCAGGCCCTCGGAGCGACCTGCTTCTTTGGGCAC
 L S L D A G L G Q A P W S D L L L W A L
 1750 1770 1790
 TGTGCTGAACAGGGCACAGATGGCCATGTACTTCTGGGAGATGGGTCCAATGCAGTTT
 L L N R A Q M A M Y F W E M G S N A V S
 1810 1830 1850
 CCTCAGCTCTGGGGCCTGTTTGCTGCTCCGGGTGATGGCACGCTTGGAGCCTGACGCTG
 S A L G A C L L L R V M A R L E F D A E
 1870 1890 1910
 AGGAGGCAGCACGGAGGAAGACCTGGCGTTCAAGTTTGAGGGGATGGGCGTTGACCTCT
 E A A R R K D L A F K F E G M G V D L F
 1930 1950 1970
 TTGGCGAGTGCTATCGCAGCAGTGAGGTGAGGGCTGCCCGCCTCCTCCTCGCTGCC
 G E C Y R S S E V R A A R L L L R R C P
 1990 2010 2030
 CGCTCTGGGGGATGCCACTTGCTCCAGCTGGCCATGCAAGCTGACGCCCGTGCCTTCT
 L W G D A T C L Q L A M Q A D A R A F F
 2050 2070 2090
 TTGCCAGGATGGGGTACAGTCTCTGCTGACACAGAAGTGGTGGGGAGATATGGCCAGCA
 A Q D G V Q S L L T Q K W W G D M A S T
 2110 2130 2150
 CTACACCCATCTGGGCCCTGGTTCTCGCCTTCTTTGGCCCTCCACTCATCTACACCCGCC
 T P I W A L V L A F F C P P L I Y T R L
 2170 2190 2210
 TCATCACCTTCAGGAATCAGAAGAGGAGCCACACGGGAGGAGCTAGAGTTTGACATGG
 I T F R K S E E E P T R E E L E F D M D
 2230 2250 2270
 ATAGTGTATTAAATGGGGAAGGGCCTGTGCGGACGGCGGACCCAGCCGAGAGACGCCGC
 S V I N G E G P V G T A D P A E K T P L
 2290 2310 2330

ON 2

18/31

Fig. 9 / continué 13

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3550          3570          3590
AGTACGAACAGCGCCTGAAAGTGTGGAGCGGGAGGTCCAGCAGTGTAGCCGCGTCTGG
Y E Q R L K V L E R E V Q Q C S R V L G
3610          3630          3650
GGTGGGTGGCCGAGGCGCTGAGCGGCTGTGCTGCTGCCCCAGGTGGGCGGCCACCCC
W V A E A L S R S A L L P P G G P P P P
3670          3690          3710
CTGACCTGCCTGGGTCCAAAGACTGAGCCCTGCTGGCGGACTTCAAGGAGAAGCCCCAC
D L P G S K D *
3730          3750          3770
AGGGGATTTTGCTCTAGAGTAGGCTCATCTGGGCGCTCGGCCCCGACCTGCTGGGCT
3790          3810          3830
TGTCCTTGAGGTGAGCCCCATGTCCATCTGGGCGACTGTCAGGACCACCTTTGGGAGTGT
3850          3870          3890
CATCCTTACAAACCACAGCATGCCCGGCTCCTCCAGAACCAAGTCCACGCTGGGAGGAT
3910          3930          3950
CAAGGCGCTGGATCCCGGCGGTATCCATCTGGAGGCTGCAGGGTCCTTGGGGTAACAGG
3970          3990          4010
GACCACAGACCCCTCACCACCTCACAGATTCTCACACTGGGGAATAAAGCCATTTTCAGA
4030
GGAAAAAAAAAAAAAAAAAAAA

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HVVPEKEQSWIPKIFKKKTCTTFIVDSTDGPGTLCQCGRPRTAHPAVAMEDAFGAAVTVWSDAHTTEKPTDAYELDFTGAG
SNFLRLSDRTDPAAVYSLVTRTWGFRAPNLVSVLGGSGGFVLQTLQDLRLRGLVRAAQSTGAWIVTGGHGTIGRHVGVAV
QMASTGGTKVAVGVAPWGVVRNRTLINPKGSFPARYRWRGDPEDGVQFPLDYNYSAEFLVDDGTHGCLGGENRFRRLRESY
QKTGVTGTDIDIFVLLLLIDGDEKMLTRIENTQAHVPCLLVAGSRGLMPGGTLEAHLAQDGDHKAQSTNQLLLPKDLSLC
SIDRKTLSYSERLAVAWNRVDIAQSELFGRDIOQRSFHLEASLMDALLNDRPEFVRLLSHGLSLGHFLTMRLAQLYSAAE
LIRNLLDQASHSAGTKAPALKGGAELRPPDVGHVRLMLLGMKCAPRYPSGGAWDPHPGCGFESMYLLSDKATSPISLDAGI
PW3DLLWALLLNRAQMAMYFWEMGSNAVSSALGACLLLRVMARLEPDAAEEAARRKDLAFKFEFGMVDLFCECYRSSEVRAAF
RRCPLNGDATCLQAMQADARAFFAQDGVQSLLTQKWWGDMASTTPIWALVLAFFCPPLIYTRLITFRKSEEEPTREELEFDM
INGEGPVTADPAEKTPLGVPRQSGRPGCCGRCGGRCLRRWFHFWGVFVTIFMGNVVSYLLFLLLSRVLLVDFQPAPPGG
LLYFWAFTLLCELRQLSGGGGSLASGGPGGHASLSQRLRLYLADSWNQCDLVALTCFLLGVGCRLTPGLYHLGRTVLCII
FTVRLLIHIFTVNRQLGFKIVIVSKMMKDVFFFLFPLGVWLVAIVGVATEGLLRPRDSDFPSILARVFRPYLQIFGQIPQEDMI
MEHSNCSSEPGFWAHPGAQAGTCVSYANWLVLVLLVIFLLVANILLVNLIAMFSYTFGKVQGNSDLYWKAQRYRLIREFF
ALAPPTIVISHLRLRLQLCRRPRSPQSSPALEHFRVYLSKEAERKLLTWESVHKENELLARARDKRESDSERLKRTSQKVI
KQLGHIREVEQRLKVLEREVQCCSBVLGWVAEALSRSALLPPGGPPPPDLPGSKD

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B.)

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10          30          50
ATCCAATGGCGGTCTTCCATCTCGAAGCTTCCCTCATGGACGCCCTGCTGAATGACCGG
70          90          110
CCTGAGTTCTGTCGCTTGTCTCATTTCCACGGCCTCAGCCTGGGCCACTTCTGACCCCG
130          150          170
ATGCGCCTGGCCCAACTCTACAGCGCGGCGCTCCAACTCGCTCATCGCAACCTTTTG
190          210          230
GACCAGGCGTCCCACAGCGCAGGCACCAAGCCCCAGCCCTAAAAGGGGGAGCTGCGGAG
250          270          290
CTCCGGCCCCCTGACGTGGGGCATGTGCTGAGGATGCTGCTGGGGAAGATGTGCGCGCCG
310          330          350
AGATGTATCTGCTCTCGGACAAGGCCACCTCGCCGCTCTCGCTGGATGCTGGCCTCGGGC
M Y L L S D K A T S P L S L D A G L G Q
370          390          410
AGGCCCCCTGGAGCGACCTGCTTCTTTGGGCACTGTTGCTGAACAGGGCACAGATGGCCA
A P W S D L L L W A L L L N R A Q M A M
430          450          470
TGTACTTCTGGGAGATGGGTTCATATGACGTTCTTCAGCTCTTGGGGCCTGTTGCTGC
Y F W E M G S N A V S S A L G A C L L L

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Fig. 9 / continuation 4

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490          510          530
TCCGGGTGATGGCACGCCTGGAGCCTGACGCTGAGGAGGCAGCACGGAGGAAAGACCTGG
  R V M A R L E P D A E E A A R R K D L A
550          570          590
CGTTCAAGTTTGGAGGGGATGGGCGTTGACCTCTTTGGCGAGTGCTATCGCAGCAGTGAGG
  F K F E G M G V D L F G E C Y R S S E V
610          630          650
TGAGGGCTGCCCGCCTCCTCCTCCGTCGCTGCCCGCTCTGGGGGGATGCCACTTGCCCTCC
  R A A R L L L R R C P L W G D A T C L Q
670          690          710
AGCTGGCCATGCAAGCTGACGCCCGTGCCTTCTTTGCCAGGATGGGGTACAGTCTCTGC
  L A M Q A D A R A F F A Q D G V Q S L L
730          750          770
TGACACAGAAGTGGTGGGAGATATGGCCAGCACTACACCCATCTGGGCCCTGGTTCTCG
  T Q K W W G D M A S T T P I W A L V L A
790          810          830
CCTTCTTTTGGCCCTCCACTCATCTACACCGCCTCATCACCTTCAGGAAATCAGAAGAGG
  F F C P P L I Y T R L I T F R K S E E E
850          870          890
AGCCACACGGGAGGAGCTAGAGTTTGACATGGATAGTGTCATTAAATGGGGAAGGGCCTG
  P T R E E L E F D M D S V I N G E G P V
910          930          950
TCGGGACGCGGACCCAGCCGAGAAGACGCGCTGGGGGTCCCGCGCCAGTCCGGCCGCTC
  G T A D P A E K T P L G V P R Q S G R P
970          990          1010
CGGGTGTCTGCGGGGGCGCTGCGGGGGGCGCGGTGCTACGCGCTGGTTCCACTTCT
  G C C G G R C G G R R C L R R W F H F W
1030          1050          1070
GGGGCGTGCGGGTGACCATCTTCATGGGCAACGTGGTCACTACCTGCTGTCTCTGCTGC
  G V P V T I F M G N V V S Y L L F L L L
1090          1110          1130
TTTTCTCGCGGGTGCTGCTCGTGGATTTCAGCCGGCGCGCGCTCCCTGGAGCTGC
  F S R V L L V D F Q P A P P G S L E L L
1150          1170          1190
TGCTCTATTCTGGGCTTTACGCTGCTGTGCGAGGAAGTGCGCCAGGGCCTGAGCGGAG
  L Y F W A F T L L C E E L R Q G L S G G
1210          1230          1250
GCGGGGCGAGCCTCGCCAGCGGGGGCCCCGGGCGCTGGCCATGCCTCACTGAGCCAGCGCC
  G G S L A S G G P G P G H A S L S Q R L
1270          1290          1310
TGCGCCTCTACCTCGCCAGACGCTGGAACCACTGCGACCTAGTGGCTCTCACCTGCTTCC
  R L Y L A D S W N Q C D L V A L T C F L
1330          1350          1370
TCCTGGGCGTGGGCTGCCGGCTGACCCCGGTTTGTACCACCTGGGCGGCACTGTCTCT
  L G V G C R L T P G L Y H L G R T V L C
1390          1410          1430
GCATCGACTTCATGGTTTTACGGTGCGGCTGCTTCACATCTTCACGGTCAACAAACAGC
  I D F M V F T V R L L H I F T V N K Q L
1450          1470          1490
TGGGGCCCAAGATCGTCATCGTGAGCAAGATGATGAAGACGTGTTCTTCTCTCTTCT
  G P K I V I V S K M K D V F F F L F F
1510          1530          1550
TCCTCGGCGTGTGGCTGGTAGCCTATGGCGTGGCCAGGAGGGGCTCTGAGGCCACGGG
  L G V W L V A Y G V A T E G L L R P R D
1570          1590          1610
ACAGTGACTTCCCAAGTATCCTGCGCCGCTCTTCTACCGTCCCTACCTGCAGATCTTCG
  S D F P S I L R R V F Y R P Y L Q I F G
1630          1650          1670
GGCAGATTCCCCAGGAGGACATGGACGTGGCCCTCATGGAGCACAGCAACTGCTCGTGG
  Q I P Q E D M D V A L M E H S N C S S E
1690          1710          1730
AGCCCGGCTTCTGGGCACACCTCCTGGGGCCAGGCGGGCACCTGCGTCTCCAGTATG

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Fig. 9 / continuation 5

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P G F W A H P P G A Q A G T C V S Q Y A
1750 1770 1790
CCAACCTGGCTGGTGGTGGCTCCTCGTCATCTTCCTGCTCGTGGCCAACATCCTGCTGG
N W L V V L L L V I F L L V A N I L L V
1810 1830 1850
TCAACTTGCTCATTGOCATGTTCAAGTTACACATTCGGCAAAGTACAGGGCAACAGCGATC
N L L I A M F S Y T F G K V Q G N S D L
1870 1890 1910
TCTACTGGAAGCGCGAGCGTTACCGCCTCATCCGGGAATTCCTACTCTCGGCCCGCGCTGG
Y W K A Q R Y R L I R E F H S R P A L A
1930 1950 1970
CCCCGCCCTTTATCGTCATCTCCCACTTGGCGCTCCTGCTCAGGCAATTGTGCAGGCGAC
P P F I V I S H L R L L L R Q L C R R P
1990 2010 2030
CCCCGGAGCCCCAGCGCTCCTCCCGGCCCTCGAGCATTTCGGGGTTACCTTTCTAAGG
R S P Q P S S P A L E H F R V Y L S K E
2050 2070 2090
AAGCCGAGCGGAAGCTGCTAACGTGGGAATCGGTGCATARGGAGAATTTCTGCTGGCAC
A E R K L L T W E S V H K E N F L L A R
2110 2130 2150
GCGCTAGGACAAAGCGGGAGAGCGACTCCGAGCGTCTGAAGCGCACGTCCTCAGAGGTGG
A R D K R E S D S E R L K R T S Q K V D
2170 2190 2210
ACTTGGCACTGAAACAGCTGGGACACATCCGCGAGTACGAACAGCGCTGAAAGTGTGG
L A L K Q L G H I R E Y E Q R L K V L E
2230 2250 2270
AGCGGGAGGTCCAGCAGTGTAGCCGCGCTCCTGGGGTGGTGGCCGAGGCCCTGAGCGCT
R E V Q Q C S R V L G W V A E A L S R S
2290 2310 2330
CTGCGCTTGCTGCCCCAGGTGGGCGGCCACCCCTGACCTGCCTGGGTCCAAGACTGAG
A L L P P G G P P P D L P G S K D *
2350 2370 2390
CCCTGCTGGCGGACTTCAAGGAGAAGCCCCACAGGGGATTTTGTCTTAGAGTAAGGCT
2410 2430 2450
CATCTGGGCTCGGCCCGCGCACCTGGTGGCTTGTCTTGAAGTGAGCCCCATGTCCAT
2470 2490 2510
CTGGGCCACTGTCAGGACCACCTTTGGGAGTGTCTCCTTACAAACCACAGCATGCCCGG
2530 2550 2570
CTCCTCCAGAACAGTCCAGCCTGGGAGGATCAAGGCTGGATCCCGGGCGGTTATCC
2590 2610 2630
ATCTGGAGGCTGCAGGGTCTTGGGGTAACAGGGACCAAGACCCCTCACCCTACAGAG
2650 2670 2690
TTCTCACACTGGGGAATAAAGCCATTTAGAGGAAAAAAAAAAAAAAAAAAAAA

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MYLLSDKATSPLSLDAGLGQAPWSDLLWALLLNRAQMAMYFWEMGSNAVSSALGACLLLRVMARLEPDAEEAARRKDLAFKFEEM
GVDLFGECEYRSSEVRAARLLLRCPWGDATCLQAMQADARAFFAQDGVQSLTQKWWGDMASSTPIWALVLAFFCPPLIYTRLI
TFRKSEEEPTREELEFDMDSVINGEGPVGTADPAEKTPLGVPRQSGRPGCCGRCGRRCLRRWFHFWGVPVTIFMGNVVSYLEFL
LLFSRVLLVDFQAPPGSLELLLYFWAFTLLCEELRQGLSGGGSLASGGPGPHASLSQRLRLYLADSWNQCDLVALTCFLLGVG
CRLTPGLYHLGRTVLCIDFMVFTVRLHIFTVNKQLGPKIVIVSKMKDVFFFLFGLVWLVAYGVATEGLLRPRSDFFPSILRRV
FYRPYLIQIFGQIPQEDMDVALMEHSNCSSEPGFWAHPGAQAGTCVSQYANWLVVLLLVIFLLVANILLVNLIIAMFSYTFGKVQG
NSDLWKAQRYRLIREFHSRPALAPPFIVISHLRLLLRQLCRRFRSPQSPSPALEHFRVYLSKEAERKLLTWESVHKENFLLARAR
DKRESDSERLKRTSQKVDLALKQLGHIREYEQRLKVLEREVQQCSRVLGWVAEALSRALLPPGGPPFPDLPGSKD

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Fig. 10

A)

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      10      30      50
ATTAAAGTTTATAAAACAGTGGCTGGATGGTTGGAGGATGCAGGTGGACAGAAGACGTGG
      M V G G C R W T E D V E
      70      90     110
AGCCTGCAGAAGTAAAGGAAAAGATGTCCTTTCGGGCAGCCAGGCTCAGCATGAGGAACA
P A E V K E K M S F R A A R L S M R N R
      130     150     170
GAAGGAATGACACTCTGGACAGCACCCGGACCCTGTACTCCAGCGCTCTCGGAGCACAG
R N D T L D S T R T L Y S S A S R S T D
      190     210     230
ACTTGTCTTACAGTGAAAGCGCCAGCTTCTACGCTGCCTTCAGGACACAGACGTGCCCAA
L S Y S E S A S F Y A A F R T Q T C P I
      250     270     290
TCATGGCTTCTTGGGACTTGGTGAATTTTATTCAAGCAAATTTTAAGAAACGAGAATGTG
M A S W D L V N F I Q A N F K K R E C V
      310     330     350
TCTTCTTTACCAAAGATTCCAAGGCCACGGAGAATGTGTGCAAGTGTGGCTATGCCCAGA
F F T K D S K A T E N V C K C G Y A Q S
      370     390     410
GCCAGCACATGGAAGGCACCCAGATCAACCAAAGTGAGAAATGGAATACAAGAAACACA
Q H M E G T Q I N Q S E K W N Y K K H T
      430     450     470
CCAAGGAATTTCTACCGACGCCTTTGGGGATATTTCAGTTTGAGACACTGGGGAAGAAAG
K E F P T D A F G D I Q F E T L G K K G
      490     510     530
GGAAGTATATACGTCTGTCTGCGACACGGACGCGGAAATCCTTTACGAGCTGCTGACCC
K Y I R L S C D T D A E I L Y E L L T Q
      550     570     590
AGCACTGGCACCTGAAAACACCCAACTGGTCAATTTCTGTGACCGGGGGCGCCAAGAAGT
H W H L K T P N L V I S V T G G A K N F
      610     630     650
TCGCCCTGAAGCGCGCATGCGCAAGATCTTCAGCCGGCTCATCTACATGCGCAGTCCA
A L K P R M R K I F S R L I Y I A Q S K
      670     690     710
AAGGTGCTTGGATTCTCACGGGAGGCACCCATTATGGCCTGATGAAGTACATCGGGGAGG
G A W I L T G G T H Y G L M K Y I G E V
      730     750     770
TGGTGAGAGATAACACCATCAGCAGGAGTTCAGAGGAGAATATTGTGGCCATTGGCATAG
V R D N T I S R S S E E N I V A I G I A
      790     810     830
CAGCTTGGGGCATGGTCTCCAACCGGGACACCTCATCAGGAATTGCGATGCTGAGGGCT
A W G M V S N R D T L I R N C D A E G Y
      850     870     890
ATTTTTTAGCCAGTACCTTATGGATGACTTCACAAGAGATCCACTGTATATCTTGGACA
F L A Q Y L M D D F T R D P L Y I L D N
      910     930     950
ACAACCACACACATTTGCTGCTCGTGGACAATGGCTGTCATGGACATCCCACTGTGGAAG
N H T H L L L V D N G C H G H P T V E A
      970     990    1010
CAAAGCTCCGGAATCAGCTAGAGAAGTATATCTCTGAGCGCACTATTCAAGATTCCAAT
K L R N Q L E K Y I S E R T I Q D S N Y
      1030    1050    1070
ATGGTGGCAAGATCCCCATTGTGTGTTTTGCCCAAGGAGGTGGAAAAGAGACTTTGAAAG
G G K I P I V C F A Q G G G K E T L K A
      1090    1110    1130
CCATCAATACCTCCATCAAAAATAAAATTCCTTGTGTGGTGGTGAAGGCTCGGGCCAGA
I N T S I K N K I P C V V V E G S G Q I
      1150    1170    1190
TCGCTGATGTGATCGCTAGCCTGGTGGAGGTGGAGGATGCCCTGACATCTTCTGCCGTCA
A D V I A S L V E V E D A L T S S A V K
      1210    1230    1250

```

Fig. 10 / continuation 1

AGGAGAGCTGGTGCCTTTTTACCCCGCACGGTGTCCCGGCTGCCTGAGGAGGAGACTG
E K L V R F L P R T V S R L P E E E T E
1270 1290 1310
AGAGTTGGATCAATGGCTCAAAGAAATTCTCGAATGTTCTCACCTATTAACAGTTATTA
S W I K W L K E I L E C S H L L T V I K
1330 1350 1370
AAATGGAAGAAGCTGGGGATGAAATTGTGAGCAATGCCATCTCCTACGCTCTATACAAAG
M E E A G D E I V S N A I S Y A L Y K A
1390 1410 1430
CCTTCAGCACCAGTGAGCAAGACAAGGATAACTGGAATGGGCAGCTGAAGCTTCTGCTGG
F S T S E Q D K D N W N G Q L K L L L E
1450 1470 1490
AGTGGAAACCAGCTGGACTTAGCCAATGATGAGATTTTCACCAATGACCGCCGATGGGAGA
W N Q L D L A N D E I F T N D R R W E K
1510 1530 1550
AGAGCAAACCGAGGCTCAGAGACACAATAATCCAGGTCACATGGCTGGAAAATGGTAGAA
S K P R L R D T I I Q V T W L E N G R I
1570 1590 1610
TCAAGGTTGAGAGCAAGATGTGACTGACGGCAAAGCCTCTTCTCATATGCTGGTGGTTC
K V E S K D V T D G K A S S H M L V V L
1630 1650 1670
TCAAGTCTGCTGACCTTCAAGAAGTCATGTTTACGGCTCTCATAAAGACAGACCCAAGT
K S A D L Q E V M F T A L I K D R P K F
1690 1710 1730
TTGTGCGCTCTTTCTGGAGAATGGCTTGAACCTAOGGAAGTTTCTCACCCTATGATGTCC
V R L F L E N G L N L R K F L T H D V L
1750 1770 1790
TCACTGAACCTCTTCTCCAACCACTTCAGCAGGCTTGTGTACCGGAATCTGCAGATCGCCA
T E L F S N H F S T L V Y R N L Q I A K
1810 1830 1850
AGAATTCCTATAATGATGCCCTCCTCACGTTTGTCTGGAAGTGGTTCGGAACCTCCGAA
N S Y N D A L L T F V W K L V A N F R R
1870 1890 1910
GAGGCTTCCGGAAGGAAGACAGAAATGGCCGGGACGAGATGGACATAGAAGTCCACGAG
G F R K E D R N G R D E M D I E L H D V
1930 1950 1970
TGCTCTCTATTACTCGGCACCCCTGCAAGCTCTCTTCATCTGGGCAATTCTTCAGAATA
S P I T R H P L Q A L F I W A I L Q N K
1990 2010 2030
AGAAGGAACCTCTCAAAGTCATTTGGGAGCAGACCAGGGGCTGCACTCTGGCAGCCCTGG
K E L S K V I W E Q T R G C T L A A L G
2050 2070 2090
GAGCCAGCAAGCTTCTGAAGACTCTGGCCAAAGTGAAGAAGACATCAATGCTGCTGGGG
A S K L L K T L A K V K N D I N A A G E
2110 2130 2150
AGTCCGAGGAGCTGGCTAATGAGTACGAGACCGGGCTGTTGGTGAGTCCACAGTGTGGA
S E E L A N E Y E T R A V G E S T V W N
2170 2190 2210
ATGCTGTGGTGGGCGCGGATCTGCCATGTGGCAGACATTGCCAGCGGCACTCATAGAC
A V V G A D L P C G T D I A S G T H R P
2230 2250 2270
CAGATGGTGGAGAGCTGTTCACTGAGTGTACAGCAGCGATGAAGACTTGGCAGAACAGC
D G G E L F T E C Y S S D E D L A E Q L
2290 2310 2330
TGCTGCTTATTCTGTGAAGCTTGGGGTGAAGCAACTGTCTGGAGCTGGCGGTGGAGG
L V Y S C E A W G G S N C L E L A V E A
2350 2370 2390
CCACAGACCAGCATTTTCATCGCCAGCCTGGGGTCCAGAATTTTCTTTCTAAGCAATGTT
T D Q H F I A Q P G V Q N F L S K Q W Y
2410 2430 2450
ATGGAGAGATTTCCCGAGACACCAAGAACTGGGAGATTATCCTGTGTCTGTTTATTATAC
G E I S R D T K N W K I I L C L F I I P

Fig. 10 / continuation 2

2470 2490 2510
CCTTGGTGGGCTGTGGCTTTGTATCATTTAGGAAGAAACCTGTCGACAAGCACAGAAGC
L V G C G F V S F R K K P V D K H K K L
2530 2550 2570
TGCTTTGGTACTATGTGGCGTTCTTACCTCCCCCTTCGTGGTCTTCTCCTGGAATGTGG
L W Y Y V A F F T S P F V V F S W N V V
2590 2610 2630
TCTTCTACATCGCCTTCTCCTGCTGTTTGCCTACGTGCTGCTCATGGATTTCCATTCGG
F Y I A F L L L F A Y V L L M D F H S V
2650 2670 2690
TGCCACACCCCCCGAGCTGGTCCGTACTCGCTGGTCTTTGTCCTTCTCTGTGATGAAG
P H P P E L V L Y S L V F V L F C D E V
2710 2730 2750
TGAGACAGGGCCGGCCGGCTGCTCCAGTGCGGGGCCCGCCAGCCACGCCACCCGGA
R Q G R P A A P S A G P A K P T P T R N
2770 2790 2810
ACTCCATCTGGCCCGCAAGCTCCACACGCAGCCCCGGTTCCCGCTCACGCCACTCCTTCC
S I W P A S S T R S P G S R S R H S F H
2830 2850 2870
ACACTTCCCTGCAAGCTGAGGGTGCCAGCTCTGGCCTTGGCCAGCCAGAAAGGGGTGGA
T S L Q A E G A S S G L G Q P R K G W T
2890 2910 2930
CATTTAAAAATCTGGAAATGGTTGATATTTCCAAGCTGCTGATGCTCCCTCTCTGTCCCTT
F K N L E M V D I S K L L M S L S V P F
2950 2970 2990
TCTGTACGCAGTGGTACGTAAATGGGGTGAATTATTTTACTGACCTGTGGAATGTGATGG
C T Q W Y V N G V N Y F T D L W N V M D
3010 3030 3050
ACACGCTGGGGCTTTTTTACTTCATAGCAGGAATTGTATTTCCGCAAGGGATCCTTAGGC
T L G L F Y F I A G I V F R Q G I L R Q
3070 3090 3110
AGAATGAGCAGCGCTGGAGGTGGATATTCGGTTCGCTCATCTACGAGCCCTACCTGGCCA
N E Q R W R W I F R S V I Y E P Y L A M
3130 3150 3170
TGTTCCGCCAGGTGCCAGTGAOGTGGATGGTACCAGTATGACTTTGCCCACTGCACCT
F G Q V P S D V D G T T Y D F A H C T F
3190 3210 3230
TCACTGGGAATGAGTCCAAGCCACTGTGTGTGGAGCTGGATGAGCACAACTGCCCGGT
T G N E S K P L C V E L D E H N L P R F
3250 3270 3290
TCCCCGAGTGGATCACCATCCCCCTGGTGTGCATCTACATGTTATCCACCAACATCCTGC
P E W I T I P L V C I Y M L S T N I L L
3310 3330 3350
TGGTCAACCTGCTGGTCCCATGTTTGGCTACACGGTGGGCACCGTCCAGGAGAACAATG
V N L L V A M F G Y T V G T V Q E N N D
3370 3390 3410
ACCAGGTCTGGAAGTCCAGAGTACTTCTGCTGCAGGAGTACTGCAGCCGCTCAATA
Q V W K F Q R Y F L V Q E Y C S R L N I
3430 3450 3470
TCCCCCTCCCCCTTCATCGTCTTCTGCTTACTTCTACATGGTGGTGAAGAAGTGTTCAGT
P F P F I V F A Y F Y M V V K K C F K C
3490 3510 3530
GTTGCTGCAAGGAGAAAAACATGGAGTCTTCTGCTGCTGTGAGTGGTTTATCCATGTGT
C C K E K N M E S S V C C E W F I H V Y
3550 3570 3590
ACTTGGGATCAGAAGCAGCGATTAAATTCAGGGAAGGATGCCTGCATCCAGTGATTGGAA
L G S E A A I N F R E G C L H P V I G S
3610 3630 3650
GCTGGACCCAGGCTGGCTGGTCTGGACATCCACACGATTCTCACATGCAGTCCCGGCT
W T P G W L V W T S T R I L T C S A G W
3670 3690 3710
GGCCAGCAGCAGGGAGTCTCAGTGTCCACACATAGCAGCTGGGTTCTTGCAAAAAGCA

Fig. 10 / continuation 3

P A A G S L S V T T H S S W V P A K S S
 3730 3750 3770
 GCAAGTCACAGGCCACCCAGACAGACGGGTAGAGAATGTGACTCTGCTTCTGGGTGGG
 K S Q A H P D R T G R E C D S A S G W E
 3790 3810 3830
 AAGGACAGCCTGCCCGGTGGGTGGAAGAATCCGTGGCCCTGTTTGGCCATCGTGGCCCTG
 G Q P A R W V E E S V A L F G H R G P V
 3850 3870 3890
 TTTGGCCACCTACCACTCTAGGCATCACTGAGCTGAATGOGCGGTCTCTGA
 W P P T T L G I T E L N A P V L *

MVGGCRWTEDEVEPAEVKEKMSFRAARLSMRNRNDTLDSTRTLYSSASRSTDLSESESAFYAAPTQTCPIMASWDLVNFQANF
 KKRECVFTKDSKATENVCKGTAQSQHMEGTQINQSEKNWYKHHKEFTDAFGDIQFETLGGKGYIRLSCTDABEILYELLTQ
 HWHLKTFNLVISTGGAKNFALKPRMRKIFSRLLIYIAQSKGAWILTGGTHYGLMKYIGEVVRDNTISRSEENIVAIGIAAWGMVS
 NRDTLIRNCDAEGYFLAQYLMDDFTDRDPLYILDNNHTHLLLDVNGCHGHFTVEAKLRNQLEKYISERTIQDSNYGGKIPIVCFAQG
 GGETLKAINTSIKKNIPCVVVEGSGQIADVIASLVEVEDALTSSAVKEKLVRFLEPRTVSRLEEBETESWIKWLKEILECSHLLTV
 IKMEEAGDEIVSNAISYALYKAFSTSEQDKDNWNGQLKLLLEWNQDLNDEIFTNDRRWEKSKPRLRDTIIQVTWLENGRIKVES
 KDVTIDGKASSEMLVVLKSADLQEVMTALIKDRPKFVRLFLENGINLRKELTHDVLTELFNHFSTLVYRNQLAKNSYNDALLTF
 VWKLVANFRGRFRKEDRNGRDEMDEIELHDVSPITRHPQLALFIWAILQNKKELSKVIWEQTRGCTLAALGASKLLKTAKVKNDIN
 AAGESEELANEYETRAVGESTVWNAVVGADLPCSTDIASGTHRPDGGELFTECYSDDEDLAEQLLVYSCEAWGGSNCLELAVEATD
 QHFLAQPGVQNFSLKQWYGEISRDTKNWKIILCLFIIPLVGCSEFVSFRKKFVDKHKLLWYVAFFTSPFVVFVSWNVVFTAFLLL
 FAYVLLMDFHVSVPHPPELVLYSLVFLFCDEVQRPAAPSAGPAKPTPTRNSIWPASSTRSPGSRSRHSFHTSLQAEAGASSGLGQ
 PRKGWTEKNLEMVDISKLLMSLSVPFCTQWYVNGVNYFTDLNWNMDTLGLFYFIAGIVFRQILRQNEQRWRWIFRSVIYEFLAM
 FGQVPSVDVDTTYDFAHCTFTGNESKPLCVELDEHNLPRFPWITIPLVCIYMLSTNILLVNLVAMFGYTVGTQENNDQVWKFP
 RYFLVQCYCSRNLNIPFPFIVFAYFYMVVKKCFKCCCKEKNMESSVCCWEFIHVYLGSEAAINFREGCLHPVIGSWTPGWLVTSTR
 ILTCSAGWPAAGSLSVTTHSSWVPKSSSKSAHPDRGTGRECDASAGWEGQPARWVEESVALFGHRGEVWPPTTLGITELNAPVL

B.

Q L
 2290 2310 2330
 TGCTGGTCTATTCTCTGAAGCTTGGGGTGGAAOCAACTGTCTGGAGCTGGCGGTGGAGG
 L V Y S C E A W G G S N C L E L A V E A
 2350 2370 2390
 CCACAGACCAGCATTTTCATGCCCCAGCCTGGGGTCCAGAATTTCTTTCTAAGCAATGGT
 T D Q H F I A Q P G V Q N F L S K Q W Y
 2410 2430 2450
 ATGGAGAGATTTCCCAGACACCAAGAAGTGAAGATTATCTGTGTCTGTTATTATAC
 G E I S R D T K N W K I I L C L F I I P
 2470 2490 2510
 CCTTGGTGGGCTGTGGCTTGTATCATTTAGGAAGAAACCTGTGCAAGCACAGAAGC
 L V G C G F V S F R K K P V D K

Figure 11:

a.) Trp10b cDNA and derived amino acid sequence

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      10      30      50
ATGAAATCCTTCCTTCCTGTCCACACCATCGTGCCTTATCAGGGAGAATGIGTGCAAGTGT
M K S F L P V H T I V L I R E N V C K C
      70      90     110
GGCTATGCCAGAGCCAGCACATGGAAGGCACCCAGATCAACCAAAGTGAGAAATGGAAC
G Y A Q S Q H M E G T Q I N Q S E K W N
     130     150     170
TACAAGAAACACACCAAGGAATTTCTACCGACGCCTTTGGGGATATTTCAGTTTGAGACA
Y K K H T K E F P T D A F G D I Q F E T
     190     210     230
CTGGGGAAGAAAGGAAGTATATACGTCTGTCTGCGACACGGACGCGSAAATCCTTTAC
L G K K G K Y I R L S C D T D A E I L Y
     250     270     290
GAGCTGTGACCCAGCACTGGCACCTGAAACACCCAACTGGTCATTCTGTGACCGGG
E L L T Q H W H L K T P N L V I S V T G
     310     330     350
GGCGCCAGAAGCTTCGCCCTGAAGCCGCGCATGCGCAAGATCTTCAGCCGGCTCATCTAC
G A K N F A L K P R M R K I F S R L I Y
     370     390     410
ATCGCGCAGTCCAAAGGTGCTTGGATTCTCACGGGAGGCACCCATTATGGCCTGATGAAG
I A Q S K G A W I L T G G T H Y G L M K
     430     450     470
TACATCGGGGAGGTGGTGAGAGATAACACCATCAGCAGGAGTTCAGAGGAGAATATTGTG
Y I G E V V R D N T I S R S S E E N I V
     490     510     530
GCCATTGGCATAGCAGCTTGGGGCATGGTCTCCAACCGGGACACCCCTCATCAGGAATTGC
A I G I A A W G M V S N R D T L I R N C
     550     570     590
GATGCTGAGGGCTATTTTTAGCCCACTACCTTATGGATGACTTCACAAGAGATCCACTG
D A E G Y F L A Q Y L M D D F T R D P L
     610     630     650
TATATCCTGGACAACAACACACACATTTGCTGCTCGTGGACAATGGCTGTCATGGACAT
Y I L D N N H T H L L L V D N G C H G H
     670     690     710
CCCCTGTGGAAGCAAAGCTCCGGAATCAGCTAGAGAAGTATATCTCTGAGCGCACTATT
P T V E A K L R N Q L E K Y I S E R T I
     730     750     770
CAAGATTCCAACCTATGGTGGCAAGATCCCCATTGTGTGTTTTGCCCAAGGAGGTGGA
Q D S N Y G G K I P I V C F A Q G G K
     790     810     830
GAGACTTTGAAAGCCATCAATACCTCCATCAAAAATAAAATTCCTTGTGTGGTGGTGGAA
E T L K A I N T S I K N K I P C V V V E
     850     870     890
GGCTCGGGCCAGATCGCTGATGTGATCGCTAGCCTGGTGGAGGTGGAGGATGCCCTGACA
G S G Q I A D V I A S L V E V E D A L T
     910     930     950
TCTTCTGCCGTCAAGGAGAAGCTGGTGGCTTTTACCCCGCACGGTGTCCCGGCTGCCT
S S A V K E K L V R F L P R T V S R L P
     970     990    1010
GAGGAGGAGACTGAGAGTTGGATCAAATGGCTCAAAGAAATTCGAAATGTTCTCACCTA
E E E T E S W I K W L K E I L E C S H L
    1030    1050    1070
TTAACAGTTATTAATAATGGAAGAAGCTGGGGATGAAATTTGTGAGCAATGCCATCTCCTAC
L T V I K M E E A G D E I V S N A I S Y
    1090    1110    1130
GCTCTATACAAAGCCTTCAGCACCAAGTGGAGCAAGACAAGGATAACTGGAATGGGCAGCTG
A L Y K A F S T S E Q D K D N W N G Q L

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Fig. 11 (Continuation)

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2410      2430      2450
AGAAACTTAGGACCCCAAGATTATAATGCTGCAGAGGATGCTGATCGATGTGTTCTTCTTC
R N L G P K I I M L Q R M L I D V F F F
2470      2490      2510
CTGTTCTCTTTTGGCGGTGTTGGATGGTGGCCTTTGGCGTGGCCAGGCAAGGGATCCTTAGG
L F L F A V W M V A F G V A R Q G I L R
2530      2550      2570
CAGAATGAGCAGCGCTGGAGGTGGATATTCCGTTCCGGTCATCTACGAGCCCTACCTGGCC
Q N E Q R W R W I F R S V I Y E F Y L A
2590      2610      2630
ATGTTCCGCCAGGTGCCAGTGACGTGGATGGTACCACGTATGACTTTGCCCACTGCACC
M F G Q V P S D V D G T T Y D F A H C T
2650      2670      2690
TTCAC TGGGAATGAGTCCAAGCCACTGTGTGTGGAGCTGGATGAGCACAACCTGCCCCGG
F T G N E S K P L C V E L D E H N L P R
2710      2730      2750
TTCCCCGAGTGGATCACCATCCCCCTGGTGTGCATCTACATGTTATCCACCAACATCCTG
F P E W I T I P L V C I Y M L S T N I L
2770      2790      2810
CTGGTCAACCTGCTGGTCCGCCATGTTTGGCTACACGGTGGGCACCGTCCAGGAGAACAAAT
L V N L L V A M F G Y T V G T V Q E N N
2830      2850      2870
GACCAGGTCTGGAAGTTCCAGAGGTACTTCTGGTGCAGGAGTACTGCAGCCGCTCAAT
D Q V W K F Q R Y F L V Q E Y C S R L N
2890      2910      2930
ATCCCCCTTCCCTTCATCGTCTTCCGCTTACTTCTACATGGTGGTGAAGAAGTGCTTCAAG
I P F P F I V F A Y F Y M V V K K C F K
2950      2970      2990
TGTTGCTGCAAGGAGAAAAACATGGAGTCTTCTGTCTGCTGTTTCAAAAATGAAGACAAT
C C C K E K N M E S S V C C F K N E D N
3010      3030      3050
GAGACTCTGGCATGGGAGGGTGTCTATGAAGGAAAACTACCTTGTCAGATCAACACAAAA
E T L A W E G V M K E N Y L V K I N T K
3070      3090      3110
GCCAACGACACCTCAGAGGAAATGAGGCATCGATTTAGACAACCTGGATACAAAGCTTAAT
A N D T S E E M R H R F R Q L D T K L N
3130      3150
GATCTCAAGGGTCTACTGAAAGAGATTGCTAATAAAATCAAATAG
D L K G L L K E I A N K I K *

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b.) Trp10 protein:

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MKSFLPVHTIVLIRENVCKCGYAQSQHMEGTQINQSEKWNKYKHTKEPPTDAFGDIQFETLGKKGKYIRLSCD TDABILY
ELLTQHWHLKTPNLVISVTGGAKNFALKPRMRKIFSR LIYIAQSKGAWILTG GTHYGLMKYIGEVVRDNTISR SSEENIV
AIGIAAWGMVSNRDTLRNCDAEGYFLAQYLMDDPTRDPL YTLDDNNHTHLLLDVNGCHGHPTVEAKLRN QLEKYISERTI
QDSNYGGKPIVCFAGGGKETLKAINTSIKNKIPCVVVEGSGQIADVIASLVEVEDALTSSAVKEKLV RFLPRTVSRLP
EBETESWIKWLKEILECSHLLTVIKMERAGDEIVSNAISYALYKAFSTSRQDKDNWNGQLKLLLEWNQDL DLANDEIFTND
RRWESADLQEVMTALIKDRPKFVRLFLENGLNLRKFLTHDVLTELF SNHPSLTVYRN LQIAKN SYNDALLTFVWKL VAN
FRRGFRKEDRNGRDEMIDELHDVSPITRHPLOALFIWAILQNKKELSKVIWEQTRGCTLAALGASKLLKTLAKVKNDINA
AGESEELANEYETRAVELFTECYSSDEDLAEQLLVYSCEAWGGSNCLELAVEATDQHFIAQPGVQNF LSKQWYGEISRDT
KNWKIILCLFIIPLVGCGFVSFRKKPVDKHKLLWYYVAFFTSPFVVFSWNVVFI AFLLLFAYVLLMDFH SVPHPELV
LYSLVFVLPFCDEV RQWYVNGVNYFTDLWNVMDTLGLFYFIAGIVFRLHSSNKSSLYSGRVIFCLDYIIIFTLRLIHIFTVS
RNLGPKIIMLQRM LIDVFFFLFLFAVVMVAFGVARQGILRQNEQRWRWIFRSVIYEPYLA MFGQVPSDVGTT YDFAHCT
FTGNESKPLCVELDEHNLPRFPEWITIPLVCIYMLSTNILLVNL LVMFGYTVGTVOENNDQVWK FQRYFLVQEYCSRLN
IPFFFI VFAYFYPMVVKCFKCCCKEKNMESSVCCFKNEDNETLAWEGVMKENYL VKINTKANDTSEMRHRFRQLD TKLN
DLKGLLKEIANKIK

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The Trp8 Gene is expressed in endometrial or uterine
in normal endometrium

Endometrial cancer:

A



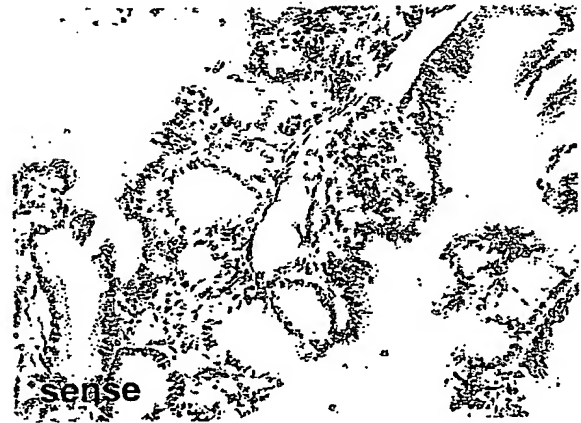
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C



D

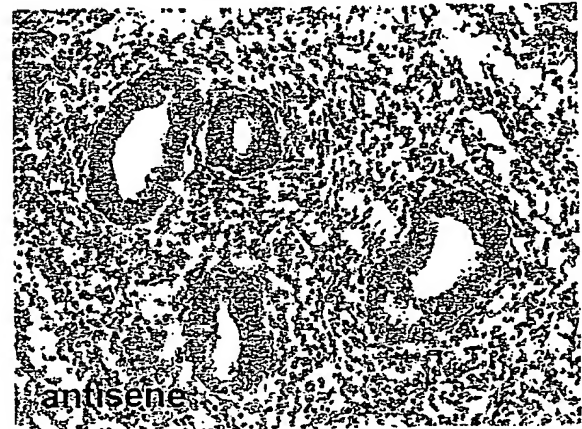


Endometrium:

E



F



Expression of human Trp 9 and Trp 10

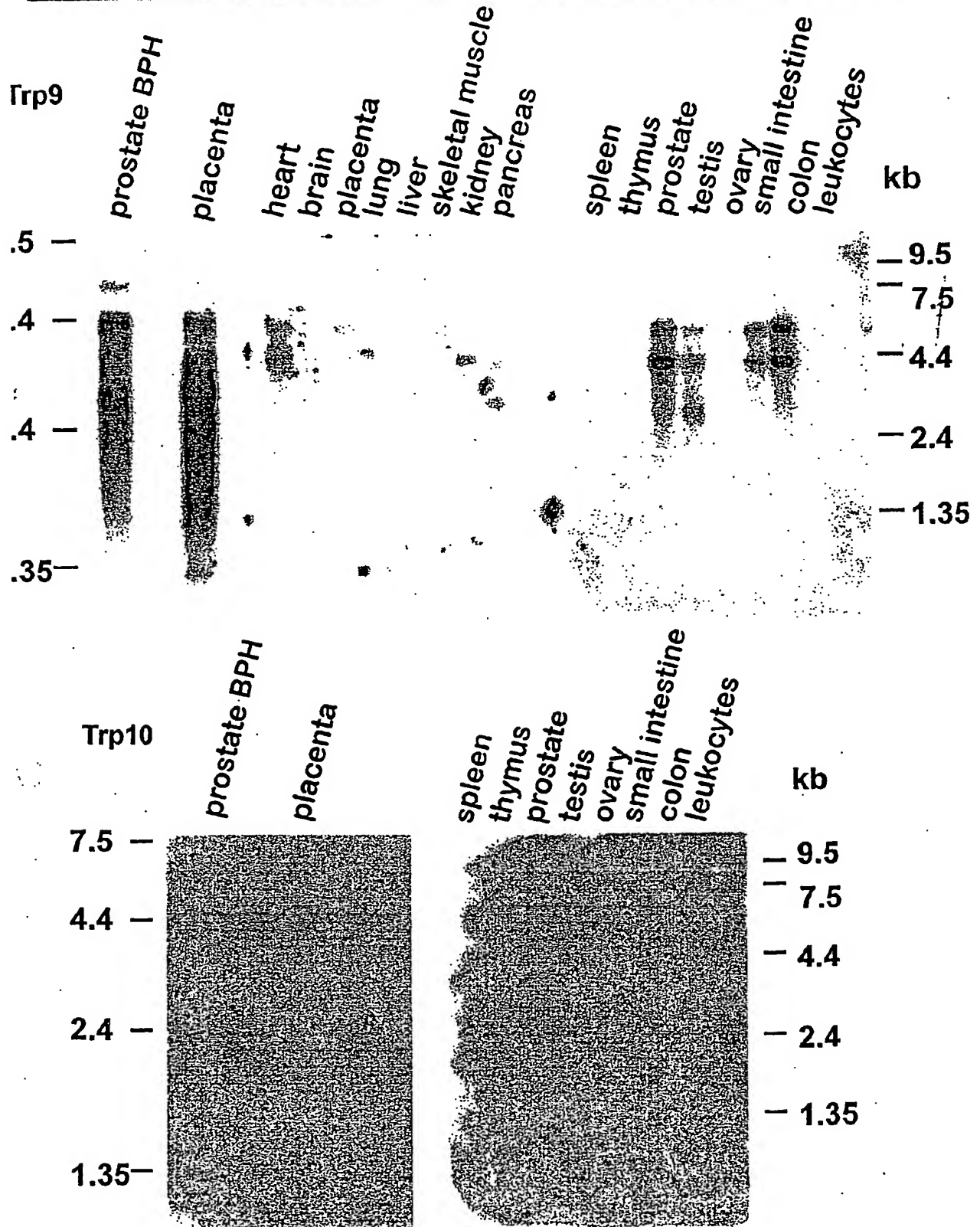
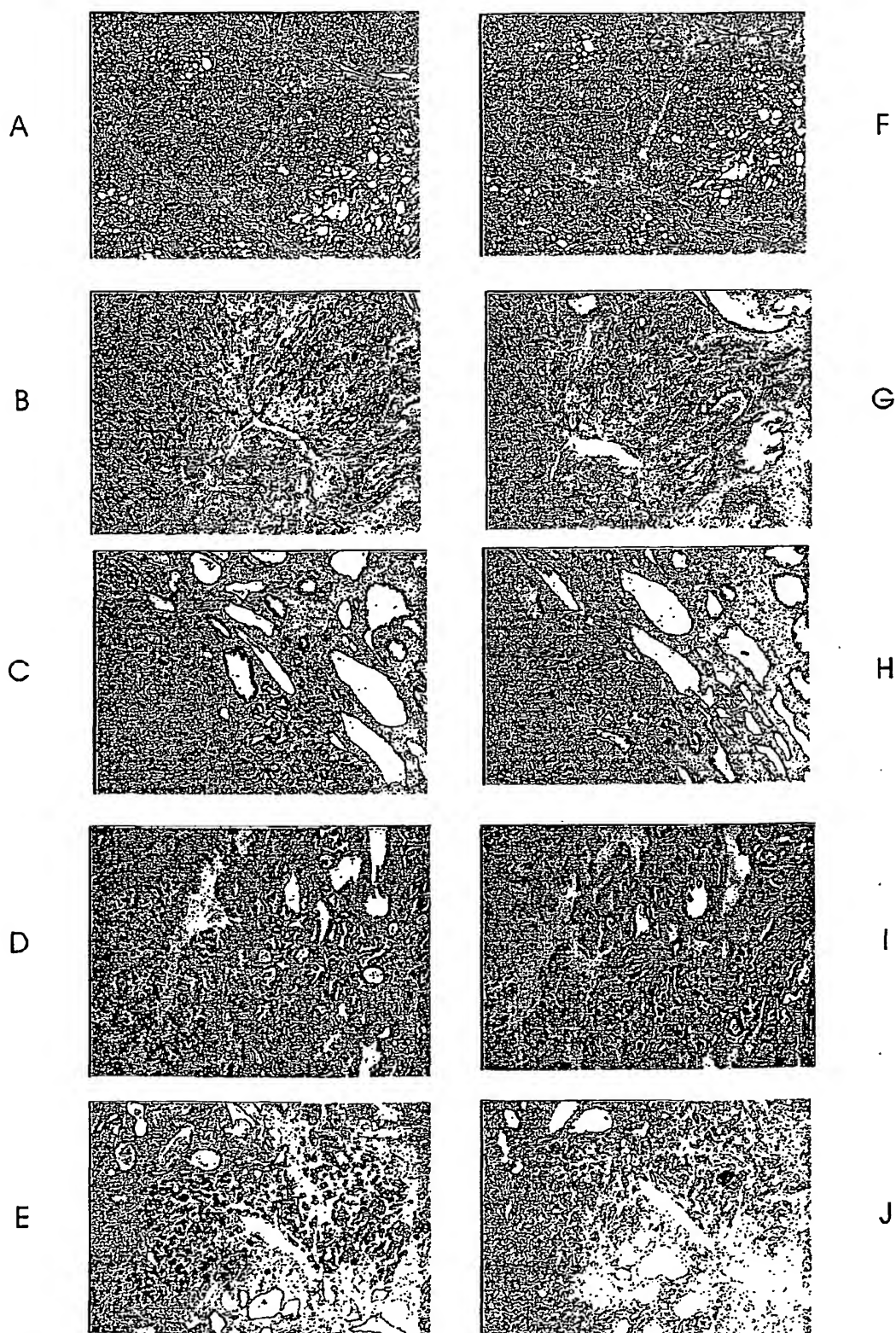


Fig. 14

Expression of Trp10 transcripts and Trp10-antisense transcripts
in human prostate cancer and in malignant melanoma



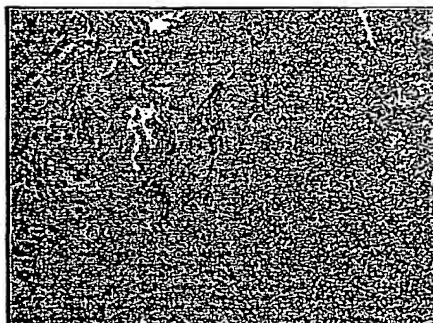
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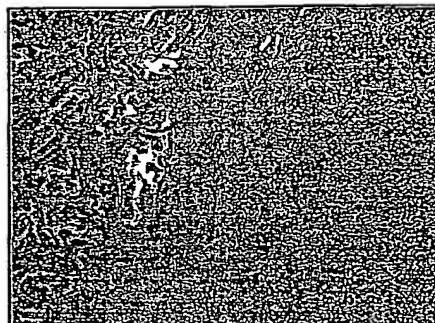
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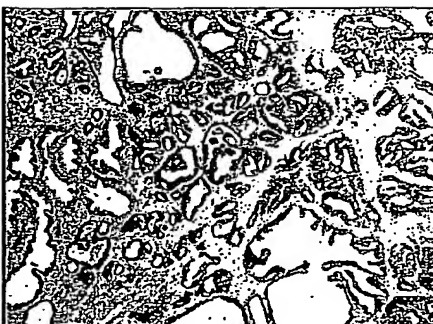
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M



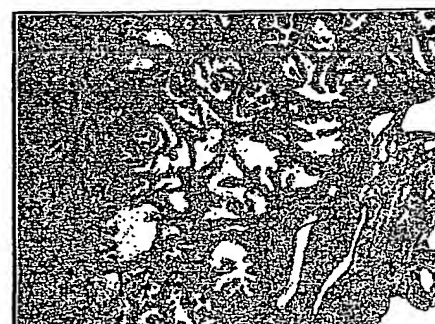
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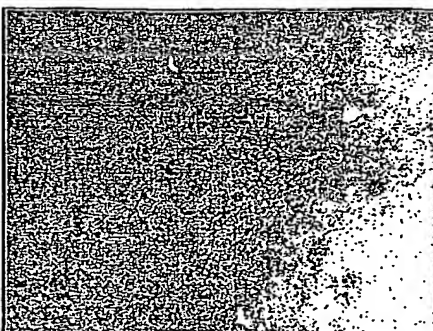
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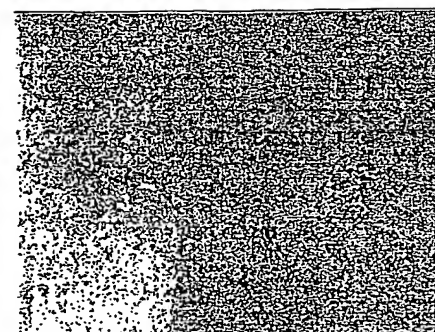
S



O



T



(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 February 2002 (07.02.2002)

PCT

(10) International Publication Number
WO 02/010382 A3

(51) International Patent Classification⁷: C12N 15/12, 15/11, 9/00, C07K 14/47, C12Q 1/68, G01N 33/577, A61K 31/713, C07K 14/705

(21) International Application Number: PCT/EP01/08309

(22) International Filing Date: 18 July 2001 (18.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/221,513 28 July 2000 (28.07.2000) US

(71) Applicant and

(72) Inventor: WISSENBACH, Ulrich [DE/DE]; Institut für Pharmakologie und Toxikologie der Uni, versität des Saarlandes, 66421 Homburg (DE).

(74) Agent: HUBER, Bernard; Huber & Schüssler, Truderinger Str. 246, 81825 München (DE).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

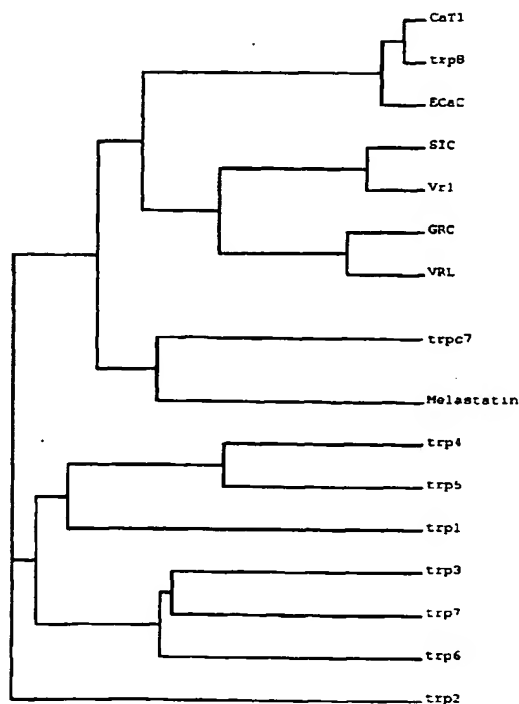
(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

[Continued on next page]

(54) Title: TRP8, TRP9 AND TRP10, MARKERS FOR CANCER



(57) Abstract: The present invention relates to gene expression in normal cells and cells of malignant tumors and particularly to novel markers associated with cancer, Trp8, Trp9 and Trp10, and the genes encoding Trp8, Trp9 and Trp10. Also provided are vectors, host cells, antibodies, and recombinant methods for producing these human proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating a tumor.



WO 02/010382 A3



(88) **Date of publication of the international search report:**
9 October 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(15) **Information about Correction:**

Previous Correction:

see PCT Gazette No. 38/2002 of 19 September 2002, Section II

INTERNATIONAL SEARCH REPORT

national Application No

PCT/EP 01/08309

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C12N15/11 C12N9/00 C07K14/47 C12Q1/68
 G01N33/577 A61K31/713 C07K14/705

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

BIOSIS, EPO-Internal, SEQUENCE SEARCH, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 09166 A (SHAPERO MICHAEL H ;DENDREON CORP (US); LAUS REINER (US); TSAVALER) 25 February 1999 (1999-02-25) see SEQID14 + 15, pages 2,3, 28,29, Example 4 table 3	1-10, 12-17, 23,29-31
X	WO 00 40614 A (BETH ISRAEL HOSPITAL ;SCHARENBERG ANDREW M (US)) 13 July 2000 (2000-07-13) see seqid31 + 32, page 11, first paragraph, page 44, lines 13-15 --- -/--	1-10,12, 31

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the International filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the International filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

a document member of the same patent family

Date of the actual completion of the International search

6 March 2003

Date of mailing of the International search report

13. 03. 2003

Name and mailing address of the ISA

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Holtorf, S

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 01/08309

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MULLER D ET AL: "Molecular cloning, tissue distribution, and chromosomal mapping of the human epithelial Ca ²⁺ channel (ECAC1)." GENOMICS, vol. 67, no. 1, 1 July 2000 (2000-07-01), pages 48-53, XP002222953 ISSN: 0888-7543 the whole document	1
X	WO 98 15657 A (ABBOTT LAB) 16 April 1998 (1998-04-16) the whole document	1-12, 29-31
X	WO 98 37093 A (CORIXA CORP) 27 August 1998 (1998-08-27) the whole document	1-12, 29-31
A	TSAVALER LARISA ET AL: "TRP-P8, a novel prostate-specific gene, is upregulated in prostate cancer and other malignancies and shares high homology with TRP calcium channel proteins." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, no. 41, March 2000 (2000-03), page 694 XP008011242 91st Annual Meeting of the American Association for Cancer Research.; San Francisco, California, USA; April 01-05, 2000, March, 2000 ISSN: 0197-016X the whole document	
A	HARTENECK C ET AL: "FROM WORM TO MAN: THREE SUBFAMILIES OF TRP CHANNELS" TRENDS IN NEUROSCIENCE, ELSEVIER, AMSTERDAM, NL, vol. 23, no. 4, April 2000 (2000-04), pages 159-166, XP001012870 ISSN: 0166-2236	
P,X	WO 01 14423 A (SMITHKLINE BEECHAM PLC) 1 March 2001 (2001-03-01) see SEQid1 + 2; see example 1	1-9,31

-/--

INTERNATIONAL SEARCH REPORT

national Application No

PCT/EP 01/08309

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WISSENBACH ULRICH ET AL: "Expression of CaT-like, a novel calcium-selective channel, correlates with the malignancy of prostate cancer." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 276, no. 22, 1 June 2001 (2001-06-01), pages 19461-19468, XP002222954 ISSN: 0021-9258 the whole document ---	1-9,13, 14, 16-19, 21-23,29
P,X	WO 01 04303 A (HEDIGER MATTHIAS A) 18 January 2001 (2001-01-18) see SEQID1 + 2 the whole document ---	1-5
P,X	WO 01 42467 A (MILLENNIUM PREDICTIVE MEDICINE) 14 June 2001 (2001-06-14) see SEQID 4615 ---	1
E	WO 01 51633 A (FANGER GARY RICHARD ;HARLOCKER SUSAN L (US); MEAGHER MADELEINE JOY) 19 July 2001 (2001-07-19) see SEQID764, example 3, claims ---	1
E	WO 02 14361 A (AGENSYS INC) 21 February 2002 (2002-02-21) see SEQID1479, examples 1-4 the whole document ---	1-10, 13-23
E	WO 02 00722 A (SILOS SANTIAGO INMACULADA ;CURTIS RORY A J (US); MILLENNIUM PHARM) 3 January 2002 (2002-01-03) see SEQID4 ---	1-5
E	WO 01 68857 A (CURTIS RORY A J ;COOK WILLIAM JAMES (US); MILLENNIUM PHARM INC (US) 20 September 2001 (2001-09-20) see SEQID1, examples ---	1-5
E	WO 01 53348 A (SQUIBB BRISTOL MYERS CO ;GAUGHAN GLEN T (US); RAMANATHAN CHANDRA S) 26 July 2001 (2001-07-26) see SEQID5 the whole document ---	1
E	WO 01 62794 A (LORA JOSE M ;CURTIS RORY A J (US); GLUCKSMANN MARIA ALEXANDRA (US)) 30 August 2001 (2001-08-30) the whole document ---	1-9
E	WO 02 30268 A (EOS BIOTECHNOLOGY INC) 18 April 2002 (2002-04-18) see SEQID53 ---	1,6
	--- -/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 01/08309

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	<p>BOEDDING MATTHIAS ET AL: "The recombinant human TRPV6 channel functions as Ca²⁺ sensor in human embryonic kidney and rat basophilic leukemia cells."</p> <p>JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 277, no. 39, 27 September 2002 (2002-09-27), pages 36656-36664, XP002222955 September 27, 2002 ISSN: 0021-9258 the whole document</p> <p>-----</p>	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 01/08309

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 24-28 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: **12 partially**
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-12, 29-31 partially, 13-28 completely

Isolated nucleic acid molecules encoding human prostate carcinom associated proteins as characterized by SEQIDs 5,45,11,3 and SEQIDs 6,46,12,4, respectively; the recombinant expression of the same in host cells; the isolated proteins as characterized by SEQIDs 6,46,12,4; antisense RNA sequence and ribozyme complementary to said nucleic acid molecules; inhibitor that can suppress the activity of said prostate carcinom associated proteins; method for diagnosing a prostate carcinoma by contacting a sample with a nucleic acid, an antibody or other reagent that reacts with the mRNA of SEQIDs 5,45,11,3; method for diagnosing endometrial cancer by contacting a target sample with a nucleic acid, an antibody or other reagent that reacts with the mRNA of SEQIDs 5,45,11,3; method for diagnosing a melanoma, chorion carcinoma, cancer of the lung and of the prostate comprising contacting a target sample with a reagent which detects antisense RNA of SEQIDs 11 and 3; method for preventing prostate tumour, endometrial cancer, chorion carcinoma or cancer of the lung comprising administering an inhibiting reagent of human prostate carcinom associated proteins; diagnostic kit containing an antibody; method for identifying an agonist or an antagonist of human prostate carcinom associated proteins.

2. Claims: 1-12, 29-31 partially

Isolated nucleic acid molecule encoding human prostate carcinom associated protein as characterized by SEQIDs 7 and SEQIDs 8, respectively; the recombinant expression of the same in host cells; the isolated protein as characterized by SEQIDs 8; antisense RNA sequence and ribozyme complementary to said nucleic acid molecule; inhibitor that can suppress the activity of said prostate carcinom associated protein; diagnostic kit containing an antibody; method for identifying an agonist or an antagonist of human prostate carcinom associated proteins.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 12 partially

Present claim 12 relates to an inhibitor which is defined by reference to a desirable characteristic or property, namely suppressing the activity of the protein of claim 6.

The claims cover all inhibitors having this characteristic or property, whereas the application provides only support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for a limited number of such inhibitors.

In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the inhibitors by reference to a result to be achieved.

Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search has been carried out for those parts of the claim 12 which appear to be clear, supported and disclosed, namely those parts relating to the Trp8/10 corresponding antibody, Trp8/10 corresponding antisense construct, a Trp8/10 corresponding ribozyme.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 01/08309

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9909166	A	25-02-1999	US 6194152 B1 AU 744875 B2 AU 9021898 A CA 2300364 A1 EP 1005549 A2 JP 2001514889 T NZ 503404 A WO 9909166 A2	27-02-2001 07-03-2002 08-03-1999 25-02-1999 07-06-2000 18-09-2001 01-03-2002 25-02-1999
WO 0040614	A	13-07-2000	AU 2055600 A CA 2360396 A1 EP 1141017 A2 JP 2002536966 T WO 0040614 A2	24-07-2000 13-07-2000 10-10-2001 05-11-2002 13-07-2000
WO 9815657	A	16-04-1998	US 5919638 A EP 0954599 A1 JP 2001523948 T WO 9815657 A1 US 6110675 A	06-07-1999 10-11-1999 27-11-2001 16-04-1998 29-08-2000
WO 9837093	A	27-08-1998	US 6261562 B1 AU 731840 B2 AU 6181898 A BR 9808881 A CN 1252837 T CZ 9903016 A3 EP 1005546 A2 HU 0002095 A2 NO 994069 A NZ 337446 A PL 335348 A1 TR 9902053 T2 US 6262245 B1 WO 9837093 A2 US 2002090372 A1 US 6465611 B1 US 6395278 B1 US 6329505 B1 US 2002022248 A1 US 2002051977 A1 US 2002193296 A1 ZA 9801585 A	17-07-2001 05-04-2001 09-09-1998 11-09-2001 10-05-2000 13-03-2002 07-06-2000 28-10-2000 22-10-1999 23-02-2001 25-04-2000 21-04-2000 17-07-2001 27-08-1998 11-07-2002 15-10-2002 28-05-2002 11-12-2001 21-02-2002 02-05-2002 19-12-2002 04-09-1998
WO 0114423	A	01-03-2001	WO 0114423 A1	01-03-2001
WO 0104303	A	18-01-2001	AU 5778600 A EP 1194546 A1 WO 0104303 A1	30-01-2001 10-04-2002 18-01-2001
WO 0142467	A	14-06-2001	AU 2074201 A WO 0142467 A2	18-06-2001 14-06-2001
WO 0151633	A	19-07-2001	AU 3447401 A AU 6158700 A WO 0104143 A2 EP 1194571 A1 EP 1261708 A2	24-07-2001 30-01-2001 18-01-2001 10-04-2002 04-12-2002

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 01/08309

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0151633	A		NO 20023402 A	29-08-2002
			WO 0151633 A2	19-07-2001
			US 2002022248 A1	21-02-2002
			US 2002051977 A1	02-05-2002
			US 2002193296 A1	19-12-2002

WO 0214361	A	21-02-2002	AU 8501801 A	25-02-2002
			WO 0214361 A2	21-02-2002

WO 0200722	A	03-01-2002	AU 7024001 A	08-01-2002
			WO 0200722 A2	03-01-2002
			US 2002156253 A1	24-10-2002

WO 0168857	A	20-09-2001	AU 4746001 A	24-09-2001
			WO 0168857 A2	20-09-2001

WO 0153348	A	26-07-2001	AU 3648201 A	31-07-2001
			EP 1252189 A2	30-10-2002
			WO 0153348 A2	26-07-2001
			US 2002072101 A1	13-06-2002

WO 0162794	A	30-08-2001	AU 3859601 A	03-09-2001
			WO 0162794 A2	30-08-2001
			US 2002142377 A1	03-10-2002

WO 0230268	A	18-04-2002	US 2002068036 A1	06-06-2002
			AU 1534502 A	22-04-2002
			WO 0230268 A2	18-04-2002

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 February 2002 (07.02.2002)

PCT

(10) International Publication Number
WO 02/010382 A2

(51) International Patent Classification⁷: C12N 15/12,
15/11, 9/00, C07K 14/47, C12Q 1/68, G01N 33/577,
A61K 31/713

LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,
ZW.

(21) International Application Number: PCT/EP01/08309

(22) International Filing Date: 18 July 2001 (18.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,
TG).

(30) Priority Data:
60/221,513 28 July 2000 (28.07.2000) US

Published:
— without international search report and to be republished
upon receipt of that report

(71) Applicant and

(72) Inventor: WISENBACH, Ulrich [DE/DE]; Institut für
Pharmakologie und Toxikologie der Uni, versität des Saar-
landes, 66421 Homburg (DE).

(48) Date of publication of this corrected version:
19 September 2002

(74) Agent: HUBER, Bernard; Huber & Schüssler, Trud-
eringer Str. 246, 81825 München (DE).

(15) Information about Correction:
see PCT Gazette No. 38/2002 of 19 September 2002, Sec-
tion II

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

WO 02/010382 A2

(54) Title: TRP8, TRP9 AND TRP10, NOVEL MARKERS FOR CANCER

(57) Abstract: The present invention relates to gene expression in normal cells and cells of malignant tumors and particularly to novel markers associated with cancer, Trp8, Trp9 and Trp10, and the genes encoding Trp8, Trp9 and Trp10. Also provided are vec-
tors, host cells, antibodies, and recombinant methods for producing these human proteins. The invention further relates to diagnostic
and therapeutic methods useful for diagnosing and treating a tumor.

Trp8, Trp9 and Trp10, novel markers for cancer**FIELD OF THE INVENTION**

The present invention relates to gene expression in normal cells and cells of malignant tumors and particularly to novel markers associated with cancer, Trp8, Trp9 and Trp10, and the genes encoding Trp8, Trp9 and Trp10

BACKGROUND OF THE TECHNOLOGY

Prostate cancer is one of the most common diseases of older men world wide. Diagnosis and monitoring of prostate cancer is difficult because of the heterogeneity of the disease. For diagnosis different grades of malignancy can be distinguished according to the Gleason-Score Diagnosis. For this diagnosis a prostate tissue sample is taken from the patient by biopsy and the morphology of the tissue is investigated. However, this approach only yields subjective results depending on the experience of the pathologist. For confirmation of these results and for obtaining an early diagnosis an additional diagnostic method can be applied which is based on the detection of a prostate specific antigen (PSA). PSA is assayed in serum samples, blood samples etc. using an anti-PSA-antibody. However, since in principle PSA is also expressed in normal prostate tissue there is a requirement for the definition of a threshold value (about 4 ng/ml PSA) in order to be able to distinguish between normal and malign prostate tissue. Unfortunately, this diagnostic method is quite insensitive and often yields false-positive results. Moreover, by using this diagnostic method any conclusions as regards the grade of malignancy, the progression of the tumor and its potential for metastasizing cannot be drawn. Thus, the use of molecular markers would be helpful to distinguish benign from malign tissue and for grading and staging prostate carcinoma, particularly for patients with metastasizing prostate cancer having a very bad prognosis.

The above discussed limitations and failings of the prior art to provide meaningful specific markers which correlate with the presence of prostate tumors, in particular metastasizing tumors, has created a need for markers which can be used diagnostically, prognostically and therapeutically over the course of this disease. The present invention fulfils such a need by the provision of Trp8, Trp9 and Trp10 and the genes encoding Trp8, Trp9 and Trp10: The genes encoding Trp8 and Trp10 are expressed in prostate carcinoma and prostatic metastasis, but

not in normal prostate, benign hyperplasia (BHP) and intraepithelial prostatic neoplasia (PIN). Furthermore, expression of Trp10 transcripts is detectable in carcinoma but not in healthy tissue of the lung, the prostate, the placenta and in melanoma.

SUMMARY OF THE INVENTION

The present invention is based on the isolation of genes encoding novel markers associated with cancer, Trp8, Trp9 and Trp10. The new calcium channel proteins Trp8, Trp9 and Trp10 are members of the trp (transient receptor potential) - family, isolated from human placenta (Trp8a and Trp8b) and humane prostate (Trp9, Trp10a and Trp10b). Trp proteins belong to a steadily growing family of Ca^{2+} selective and non selective ion channels. In the recent years seven Trp proteins (trp1 - trp7) have been identified and suggested to be involved in cation entry, receptor operated calcium entry and pheromone sensory signaling. Structurally related to the trp proteins are the vanilloid receptor (VR1) and the vanilloid like receptor (VRL-1) both involved in nociception triggered by heat. Furthermore, two calcium permeable channels were identified in rat small intestine (CaT1) and rabbit kidney (ECaC). These distantly related channels are suggested to be involved in the uptake of calcium ions from the lumen of the small intestine (CaT1) or in the reuptake of calcium ions in the distal tubule of the kidney (ECaC). Common features of the Trp and related channels are a proposed structure comprising six transmembrane domains including several conserved amino acid motifs. In the present invention the cloning and expression of a CaT1 like calcium channel (Trp8) from human placenta as well as Trp9 and Trp10 (two variants, Trp10a and Trp10b) is described. Two polymorphic variants of the Trp8 cDNA were isolated from placenta (Trp8a and Trp8b). Transient expression of the Trp8b cDNA in HEK (human embryonic kidney) cells results in cytosolic calcium overload implicating that the Trp8 channel is constitutive open in the expression system. Trp8 induces highly calcium selective inward currents in HEK cells. The C-terminus of the Trp8 protein binds calmodulin in a calcium dependent manner. The Trp9 channel is expressed in trophoblasts and syncytiotrophoblasts of placenta and in pancreatic acinar cells. Furthermore, the Trp8 channel is expressed in prostatic carcinoma and prostatic metastases, but not in normal tissue of the prostate. No expression of Trp8 transcripts is detectable in benign prostatic hyperplasia (BPH) or prostatic intraepithelial neoplasia (PIN). Therefore, the Trp8 channel is exclusively expressed in malign prostatic tissues and serves as molecular marker for prostate cancer. From the experimental results it is also apparent that the

modulation of Trp8 and/or Trp10, e.g. the inhibition of expression or activity, is of therapeutic interest, e.g. for the prevention of tumor progression.

The present invention, thus, provides a Trp8, Trp9 and Trp10 protein, respectively, as well as nucleic acid molecule encoding the protein and, moreover, an antisense RNA, a ribozyme and an inhibitor, which allow to inhibit the expression or the activity of Trp8, Trp9 and/or Trp10.

In one embodiment, the present invention provides a diagnostic method for detecting a prostate cancer or endometrial cancer (cancer of the uterus) associated with Trp8 or Trp10 in a tissue of a subject, comprising contacting a sample containing Trp8 and/or Trp10 encoding mRNA with a reagent which detects Trp8 and/or Trp10 or the corresponding mRNA.

In a further embodiment, the present invention provides a diagnostic method for detecting a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense transcripts or Trp10a and/or Trp10b related antisense transcripts.

In another embodiment, the present invention provides a method of treating a prostate tumor, carcinoma of the lung, carcinoma of the placenta (chorion carcinoma) or melanoma associated with Trp8 and/or Trp10, comprising administering to a subject with such an disorder a therapeutically effect amount of a reagent which modulates, e.g. inhibits, expression of Trp8 and/or Trp10 or the activity of the protein, e.g. the above described compounds.

Finally, the present invention provides a method of gene therapy comprising introducing into cells of a subject an expression vector comprising a nucleotide sequence encoding the above mentioned antisense RNA or ribozyme, in operable linkage with a promoter.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: A, phylogenetic relationship of trp and related proteins. B, hydropathy plot of the Trp8 protein sequence according to Kyte and Doolittle. C, alignment of Trp8a/b to the epithelial calcium channels ECaC (from rabbit) and Vr1 (from rat). Putative transmembrane domains are underlined.

Figure 2: A, polymorphism of the Trp8 gene. The polymorphic variants Trp8a and Trp8b differ in five base pairs resulting in three amino acid exchanges in the derived protein sequences. Specific primers were derived from the Trp8 gene as indicated by arrows. B, the Trp8a and Trp8b genes are distinguishable by a single restriction site. Genomic fragments of the Trp8 gene can be amplified using specific primers (shown in A). The genomic fragment of the Trp8b gene contains an additional site of the restriction enzyme BSP1286I (B). C, the Trp8 gene is located on chromosome 7. D, genotyping of eleven human subjects. A 458 bp genomic fragment of the Trp8 gene was amplified using specific primers (shown in A) and restricted with BSP1286I. The resulting fragments were analyzed by PAGE electrophoresis.

Figure 3: The Trp8b protein is a calcium selective ion channel. A, representative trace of a pdiTrp8b transfected HEK 293 cell. Trp8b mediated currents are activated by voltage ramps (-100 mV - +100 mV) of 100 msec at -40 mV or +70 mV holding potential. 1, Trp8b currents in the presence at 2mM $[Ca^{2+}]_o$; 2, effect of solution switch alone 3, switch to nominal zero calcium solution. B, Trp8b currents in the presence of zero divalent cations. C, current voltage relationship of the currents shown in A. Inset, leak subtracted current. D, current voltage relationship of the current shown in B. E, statistics of representative experiments. Black: Trp8 transfected cells, gray: control cells. Columns from left to right: Trp8 currents at -40 mV (n=12) and +70 mV holding potential (n=12). Trp8 currents in standard bath solution including 120 mM NMDG without sodium (n=7) and with nominal zero calcium ions (n=8) or in the presence of 1mM EGTA with zero divalent cations (n=6). F, representative changes in $[Ca^{2+}]_i$ in Trp8b transfected HEK cells (gray) and controls (black) in the presence or absence of 1mM $[Ca^{2+}]_o$. Inset, relative increase of cytosolic calcium concentration of Trp8b transfected HEK cells, before and after readdition of 1 mM $[Ca^{2+}]_o$ in comparison to control cells.

Figure 4: The C-terminal region of the Trp8 protein binds calmodulin. A, N- and C-terminal fragments of the Trp8 protein used for calmodulin binding studies. B, the Trp8 protein and a truncated Trp8 protein which was in vitro translated after MunI cut of the cDNA, which lacks the C-terminal 32 amino acid residues, were in vitro translated in the presence of ^{35}S -methionine and incubated with calmodulin coupled agarose beads in the presence of 1 mM Ca^{2+} or 2 mM EGTA. C, calmodulin binding to N- and C-terminal fragments of the Trp8 protein in the presence of Ca^{2+} (1 mM) or EGTA (2 mM)

Figure 5: Expression pattern of the Trp8 cDNA. A, Northern blots (left panels, Clontech, Palo Alto) were hybridized using a 348 bp NcoI/BamHT fragment of the Trp9 cDNA. The probe hybridizes to mRNA species isolated from the commercial blot, but not to mRNA species isolated from benign prostate hyperplasia (right panel, mRNA isolated from 20 human subjects with benign prostate hyperplasia). B,C, in situ hybridization with biotinylated Trp8 specific oligonucleotides on slides of human tissues. Left column antisense probes, right column sense probes. D, antinsense probes.

Figure 6: Differential expression of Trp8 cDNA in human prostate. A - F, in situ hybridization with prostatic tissues. A, normal prostate, B, primary carcinoma, C, benign hyperplasia, D, rezidive carcinoma, E, prostatic intraepithelial neoplasia, F, lymphnode metastasis of the prostata.

Figure 7: Trp8a cDNA sequence and derived amino acid sequence

Figure 8: A, Trp8b cDNA sequence and derived amino acid sequence

B, cDNA sequence of splice variant 1 (12B1)

C, cDNA sequence of splice variant 2 (17-3)

D, cDNA sequence of splice variant 3 (23A3)

E, cDNA sequence of splice variant 4 (23C3)

Figure 9: A, Trp9 cDNA sequence and derived amino acid sequence B, cDNA sequence of splice variant 15 and derived amino acid sequence. .

Figure 10: A, cDNA sequence of Trp10a and derived amino acid sequence, B, cDNA fragment of Trp10a and derived amino acid sequence.

Figure 11: cDNA sequence of Trp10b and derived amino acid sequence.

Figure 12: Expression of Trp8 mRNA in human endometrial cancer or cancer of the uterus. A - D, in situ hybridization with slides of endometrial cancer hybridized with Trp8 antisense (left column) or sense probes as controls (right column). E - F, Trp8 antisense probes hybridized to slides of normal endometrium. It can be clearly seen no hybridization occurs with normal endometrial tissue.

Figure 13: Expression of human Trp9 and Trp10 genes

Northern blots were hybridized using Trp9 (upper panel) or Trp10 (lower panel) specific probes. Expression of the Trp9 cDNA is detectable in many tissues including human prostate and colon as well as in benign prostatic hyperplasia. Expression of Trp10 cDNA is detectable in human prostate of a commercial northern blot (Clontech, right side). This Northern blot contains prostatic tissue collected from 15 human subjects in the range of 14 - 60 years of age. No expression of Trp10 cDNA was detectable in benign prostatic hyperplasia (left side).

Figure 14: Expression of Trp10 transcripts and Trp10-antisense transcripts in human prostate cancer and metastasis of a melanoma. In situ hybridizations of slides hybridized with Trp10-antisense (A-E, K-N) and Trp10 related sense probes (F-J, P-R). It can clearly be seen that both probes detect the same cancer cells indicating that these cancer cells express Trp10 transcripts as well as Trp10-antisense transcripts. S, no Trp10 expression is detectable in benign hyperplasia of the prostate (BPH). O and T, show expression of Trp10 transcripts (O) and Trp10-antisense transcripts (T) in a metastasis of a melanoma in human lung. Melanoma cancer cells express both Trp10 transcripts and Trp10-antisense transcripts.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b or a protein exhibiting biological properties of Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being selected from the group consisting of

- (a) a nucleic acid molecule encoding a protein that comprises the amino acid sequence depicted in Figure 7, 8A, 9,10 or 11;
- (b) a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9,10, or 11;
- (c) a nucleic acid molecule included in DSMZ Deposit no. DSM 13579 (deposit date: 28 June 2000), DSM 13580 (deposit date: 28 June 2000), DSM 13584 (deposit date: 5 July 2000), DSM 13581 (deposit date: 28 June 2000) or DSM(deposit date:....);
- (d) a nucleic acid molecule which hybridizes to a nucleic acid molecule specified in (a) to (c)

- (e) a nucleic acid molecule the nucleic acid sequence of which deviates from the nucleic sequences specified in (a) to (d) due to the degeneration of the genetic code; and
- (f) a nucleic acid molecule, which represents a fragment, derivative or allelic variation of a nucleic acid sequence specified in (a) to (e).

As used herein, a protein exhibiting biological properties of Trp8a, Trp8b, Trp9, Trp10a or Trp10b is understood to be a protein having at least one of the activities as illustrated in the Examples, below.

As used herein, the term „isolated nucleic acid molecule,, includes nucleic acid molecules substantially free of other nucleic acids, proteins, lipids, carbohydrates or other materials with which it is naturally associated.

In a first embodiment, the invention provides an isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b comprising the amino acid sequence depicted in Figure 7, 8A, 9, 10 or 11. The present invention also provides a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9, 10 or 11.

The present invention provides not only the generated nucleotide sequence identified in Figure 7, 8A, 9, 10 or 11, respectively and the predicted translated amino acid sequence, respectively, but also plasmid DNA containing a Trp8a cDNA deposited with the DSMZ, under DSM 13579, a Trp8b cDNA deposited with the DSMZ, under DSM 13580, a Trp9 cDNA deposited with the DSMZ, under DSM 13584, a Trp10a cDNA deposited with the DSMZ, under DSM 13581, and a Trp10b cDNA deposited with the DSMZ, under DSM...., respectively. The nucleotide sequence of each deposited Trp-clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by each deposited clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited Trp-encoding DNA, collecting the protein, and determining its sequence.

The nucleic acid molecules of the invention can be both DNA and RNA molecules. Suitable DNA molecules are, for example, genomic or cDNA molecules. It is understood that all

nucleic acid molecules encoding all or a portion of Trp8a, Trp8b, Trp9, Trp10a or Trp10b are also included, as long as they encode a polypeptide with biological activity. The nucleic acid molecules of the invention can be isolated from natural sources or can be synthesized according to known methods.

The present invention also provides nucleic acid molecules which hybridize to the above nucleic acid molecules. As used herein, the term „hybridize,, has the meaning of hybridization under conventional hybridization conditions, preferably under stringent conditions as described, for example, in Sambrook et al., *Molecular Cloning, A Laboratory Manual* 2nd edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Also contemplated are nucleic acid molecules that hybridize to the Trp nucleic acid molecules at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency), salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 9.2M NaH₂PO₄; 0.02M EDTA, pH7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA, following by washes at 50°C with 1 X SSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC). Variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Nucleic acid molecules that hybridize to the molecules of the invention can be isolated, e.g., from genomic or cDNA libraries that were produced from human cell lines or tissues. In order to identify and isolate such nucleic acid molecules the molecules of the invention or parts of these molecules or the reverse complements of these molecules can be used, for example by means of hybridization according to conventional methods (see, e.g., Sambrook et al., *supra*). As a hybridization probe nucleic acid molecules can be used, for example, that have exactly or basically the nucleotide sequence depicted in Figure 7, 8A, 9, 10 or 11, respectively, or parts of these sequences. The fragments used as hybridization probe can be synthetic

fragments that were produced by means of conventional synthetic methods and the sequence of which basically corresponds to the sequence of a nucleic acid molecule of the invention.

The nucleic acid molecules of the present invention also include molecules with sequences that are degenerate as a result of the genetic code.

In a further embodiment, the present invention provides nucleic acid molecules which comprise fragments, derivatives and allelic variants of the nucleic acid molecules described above encoding a protein of the invention. „Fragments,, are understood to be parts of the nucleic acid molecules that are long enough to encode one of the described proteins. These fragments comprise nucleic acid molecules specifically hybridizing to transcripts of the nucleic acid molecules of the invention. These nucleic acid molecules can be used, for example, as probes or primers in the diagnostic assay and/or kit described below and, preferably, are oligonucleotides having a length of at least 10, in particular of at least 15 and particularly preferred of at least 50 nucleotides. The nucleic acid molecules and oligonucleotides of the invention can also be used, for example, as primers for a PCR reaction. Examples of particular useful probes (primers) are shown in Tables 1 and 2.

Table 1

Trp8 probes used for in situ hybridization:

Probes (antisense)

- 1.) 5' TCCGCTGCCGGTTGAGATCTTGCC 3'
- 2.) 5' CTTGCTCCATAGGCAGAGAATTAG 3'
- 3.) 5' ATCCTCAGAGCCCCGGGTGTGGAA3'

Controls (sense)

- 1.) 5' GGCAAGATCTCAACCGGCAGCGGA 3'
- 2.) 5' CTAATTCTCTGCCTATGGAGCAAG 3'
- 3.) 5' TTCCACACCCGGGGCTCTGAGGAT 3'

Tabelle 2

Trp10 probes used for the in situ hybridizations shown in Figure 14:

Probes (antisense)

- 1.) 5' GCTTCCACCCCAAGCTTCACAGGAATAGA 3' (Figure 14 A, 14B)
- 2.) 5' GGCGATGAAATGCTGGTCTGTGGC 3' (Figure 14C, 14D, 14N, 14S, 14O)
- 3.) 5' ATCTTCCAGTTCTTGGTGTCTCGG 3' (Figure 14E, 14K)
- 4.) 5' GCTGCAGTACTCCTGCACCAGGAA 3' (Figure 14L, 14M)

Probes (sense)

- 1.) 5' TCTATTCCTGTGAAGCTTGGGGTGGGAAGC 3' (Figure 14F, 14G)
- 2.) 5' GCCACAGACCAGCATTTTCATCGCC 3' (Figure 14H, 14I, 14T)
- 3.) 5' CCGAGACACCAAGAACTGGAAGAT 3' (Figure 14J, 14P)
- 4.) 5' TTCCTGGTGCAGGAGTACTGCAGC 3' (Figure 14Q, 14R)

The term „derivative,, in this context means that the sequences of these molecules differ from the sequences of the nucleic acid molecules described above at one or several positions but have a high level of homology to these sequences. Homology hereby means a sequence identity of at least 40%, in particular an identity of at least 60%, preferably of more than 80% and particularly preferred of more than 90%. These proteins encoded by the nucleic acid molecules have a sequence identity to the amino acid sequence depicted in Figure 7, 8A, 9, 10 and 11, respectively, of at least 80%, preferably of 85% and particularly preferred of more than 90%, 97% and 99%. The deviations to the above-described nucleic acid molecules may have been produced by deletion, substitution, insertion or recombination. The definition of the derivatives also includes splice variants, e.g. the splice variants shown in Figures 8B to 8E and 9B.

The nucleic acid molecules that are homologous to the above-described molecules and that represent derivatives of these molecules usually are variations of these molecules that represent modifications having the same biological function. They can be naturally occurring variations, for example sequences from other organisms, or mutations that can either occur naturally or that have been introduced by specific mutagenesis. Furthermore the variations can be synthetically produced sequences. The allelic variants can be either naturally occurring variants or synthetically produced variants or variants produced by recombinant DNA processes.

Generally, by means of conventional molecular biological processes it is possible (see, e.g., Sambrook et al., supra) to introduce different mutations into the nucleic acid molecules of the invention. As a result Trp proteins or Trp related proteins with possibly modified biological properties are synthesized. One possibility is the production of deletion mutants in which nucleic acid molecules are produced by continuous deletions from the 5'- or 3'-terminal of the coding DNA sequence and that lead to the synthesis of proteins that are shortened accordingly. Another possibility is the introduction of single-point mutation at positions where a modification of the amino acid sequence influences, e.g., the ion channel properties or the regulations of the trp-ion channel. By this method muteins can be produced, for example, that possess a modified ion conducting pore, a modified K_m -value or that are no longer subject to the regulation mechanisms that normally exist in the cell, e.g. with regard to allosteric regulation or covalent modification. Such muteins might also be valuable as therapeutically useful antagonists of Trp8a, Trp8b, Trp9, Trp10a or Trp10b, respectively.

For the manipulation in prokaryotic cells by means of genetic engineering the nucleic acid molecules of the invention or parts of these molecules can be introduced into plasmids allowing a mutagenesis or a modification of a sequence by recombination of DNA sequences. By means of conventional methods (cf. Sambrook et al., supra) bases can be exchanged and natural or synthetic sequences can be added. In order to link the DNA fragments with each other adapters or linkers can be added to the fragments. Furthermore, manipulations can be performed that provide suitable cleavage sites or that remove superfluous DNA or cleavage sites. If insertions, deletions or substitutions are possible, in vitro mutagenesis, primer repair, restriction or ligation can be performed. As analysis method usually sequence analysis, restriction analysis and other biochemical or molecular biological methods are used.

The proteins encoded by the various variants of the nucleic acid molecules of the invention show certain common characteristics, such as ion channel activity, molecular weight, immunological reactivity or conformation or physical properties like the electrophoretical mobility, chromatographic behavior, sedimentation coefficients, solubility, spectroscopic properties, stability, pH optimum, temperature optimum.

The invention furthermore relates to vectors containing the nucleic acid molecules of the invention. Preferably, they are plasmids, cosmids, viruses, bacteriophages and other vectors

usually used in the field of genetic engineering. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in mammalian cells and baculovirus-derived vectors for expression in insect cells. Preferably, the nucleic acid molecule of the invention is operatively linked to the regulatory elements in the recombinant vector of the invention that guarantee the transcription and synthesis of an RNA in prokaryotic and/or eukaryotic cells that can be translated. The nucleotide sequence to be transcribed can be operably linked to a promoter like a T7, metallothionein I or polyhedrin promoter.

In a further embodiment, the present invention relates to recombinant host cells transiently or stable containing the nucleic acid molecules or vectors or the invention. A host cell is understood to be an organism that is capable to take up *in vitro* recombinant DNA and, if the case may be, to synthesize the proteins encoded by the nucleic acid molecules of the invention. Preferably, these cells are prokaryotic or eukaryotic cells, for example mammalian cells, bacterial cells, insect cells or yeast cells. The host cells of the invention are preferably characterized by the fact that the introduced nucleic acid molecule of the invention either is heterologous with regard to the transformed cell, i.e. that it does not naturally occur in these cells, or is localized at a place in the genome different from that of the corresponding naturally occurring sequence.

A further embodiment of the invention relates to isolated proteins exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being encoded by the nucleic acid molecules of the invention, as well as to methods for their production, whereby, e.g., a host cell of the invention is cultivated under conditions allowing the synthesis of the protein and the protein is subsequently isolated from the cultivated cells and/or the culture medium. Isolation and purification of the recombinantly produced proteins may be carried out by conventional means including preparative chromatography and affinity and immunological separations involving affinity with an anti-Trp8a-, anti-Trp8b-, anti-Trp9-, anti-Trp10a- or anti-Trp10b-antibody, respectively.

As used herein, the term „isolated protein,, includes proteins substantially free of other proteins, nucleic acids, lipids, carbohydrates or other materials with which it is naturally associated. Such proteins however not only comprise recombinantly produced proteins but include isolated naturally occurring proteins, synthetically produced proteins, or proteins

produced by a combination of these methods. Means for preparing such proteins are well understood in the art. The Trp proteins are preferably in a substantially purified form. A recombinantly produced version of a human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b protein, including the secreted protein, can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67; 31-40 (1988).

In a further preferred embodiment, the present invention relates to an antisense RNA sequence characterised that it is complementary to an mRNA transcribed from a nucleic acid molecule of the present invention or a part thereof and can selectively bind to said mRNA, said sequence being capable of inhibiting the synthesis of the protein encoded by said nucleic acid molecules, and a ribozyme characterised in that it is complementary to an mRNA transcribed from a nucleic acid molecule of the present invention or a part thereof and can selectively bind to and cleave said mRNA, thus inhibiting the synthesis of the proteins encoded by said nucleic acid molecules. Ribozymes which are composed of a single RNA chain are RNA enzymes, i.e. catalytic RNAs, which can intermolecularly cleave a target RNA, for example the mRNA transcribed from one of the Trp genes. It is now possible to construct ribozymes which are able to cleave the target RNA at a specific site by following the strategies described in the literature. (see, e.g., Tanner et al., in: *Antisense Research and Applications*, CRC Press Inc. (1993), 415-426). The two main requirements for such ribozymes are the catalytic domain and regions which are complementary to the target RNA and which allow them to bind to its substrate, which is a prerequisite for cleavage. Said complementary sequences, i.e., the antisense RNA or ribozyme, are useful for repression of Trp8a-, Trp8b, Trp9-, Trp10a- and Trp10b-expression, respectively, i.e. in the case of the treatment of a prostate cancer or endometrial cancer (carcinoma of the uterus). Preferably, the antisense RNA and ribozyme of the invention are complementary to the coding region. The person skilled in the art provided with the sequences of the nucleic acid molecules of the present invention will be in a position to produce and utilise the above described antisense RNAs or ribozymes. The region of the antisense RNA and ribozyme, respectively, which shows complementarity to the mRNA transcribed from the nucleic acid molecules of the present invention preferably has a length of at least 10, in particular of at least 15 and particularly preferred of at least 50 nucleotides.

In still a further embodiment, the present invention relates to inhibitors of Trp8a, Trp8b, Trp9, Trp10a and Trp10b, respectively, which fulfill a similar purpose as the antisense RNAs or

ribozymes mentioned above, i.e. reduction or elimination of biologically active Trp8a, Trp8b, Trp9, Trp10a or Trp10b molecules. Such inhibitors can be, for instance, structural analogues of the corresponding protein that act as antagonists. In addition, such inhibitors comprise molecules identified by the use of the recombinantly produced proteins, e.g. the recombinantly produced protein can be used to screen for and identify inhibitors, for example, by exploiting the capability of potential inhibitors to bind to the protein under appropriate conditions. The inhibitors can, for example, be identified by preparing a test mixture wherein the inhibitor candidate is incubated with Trp8a, Trp8b, Trp9, Trp10a or Trp10b, respectively, under appropriate conditions that allow Trp8a, Trp8b, Trp9, Trp10a or Trp10b to be in a native conformation. Such an in vitro test system can be established according to methods well known in the art. Inhibitors can be identified, for example, by first screening for either synthetic or naturally occurring molecules that bind to the recombinantly produced Trp protein and then, in a second step, by testing those selected molecules in cellular assays for inhibition of the Trp protein, as reflected by inhibition of at least one of the biological activities as described in the examples, below. Such screening for molecules that bind Trp8a, Trp8b, Trp9, Trp10a or Trp10b could easily be performed on a large scale, e.g. by screening candidate molecules from libraries of synthetic and/or natural molecules. Such an inhibitor is, e.g., a synthetic organic chemical, a natural fermentation product, a substance extracted from a microorganism, plant or animal, or a peptide. Additional examples of inhibitors are specific antibodies, preferably monoclonal antibodies. Moreover, the nucleic acid sequences of the invention and the encoded proteins can be used to identify further factors involved in tumor development and progression. In this context it should be emphasized that the modulation of the calcium channel of a member of the trp family can result in the stimulation of the immune response of T lymphocytes leading to proliferation of the T lymphocytes. The proteins of the invention can, e.g., be used to identify further (unrelated) proteins which are associated with the tumor using screening methods based on protein/protein interactions, e.g. the two-hybrid-system Fields, S. and Song, O. (1989) *Nature* (340): 245-246.

The present invention also provides a method for diagnosing a prostate carcinoma which comprises contacting a target sample suspected to contain the protein Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA with a reagent which reacts with Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA and detecting Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA.

It has been found that carcinoma cells of placenta (chorion carcinoma), lung and prostate express Trp10 transcripts as well as Trp10 antisense transcripts and transcripts being in part complementary to Trp10 antisense transcripts. Accordingly, the present invention also provides a method for diagnosing a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense RNA.

When the target is mRNA (or antisense RNA), the reagent is typically a nucleic acid probe or a primer for PCR. The person skilled in the art is in a position to design suitable nucleic acids probes based on the information as regards the nucleotide sequence of Trp8a, Trp8b, Trp10a or Trp10b as depicted in figure 7, 8a, 10 and 11, respectively, or tables 1 and 2, above. When the target is the protein, the reagent is typically an antibody probe. The term „antibody“, preferably, relates to antibodies which consist essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations. Monoclonal antibodies are made from an antigen containing fragments of the proteins of the invention by methods well known to those skilled in the art (see, e.g., Köhler et al., *Nature* 256 (1975), 495). As used herein, the term „antibody“ (Ab) or „monoclonal antibody“ (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')₂ fragments) which are capable of specifically binding to protein. Fab and f(ab')₂ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. (Wahl et al., *J. Nucl. Med.* 24: 316-325 (1983)). Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimerical, single chain, and humanized antibodies. The target cellular component, i.e. Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts, e.g., in biological fluids or tissues, may be detected directly in situ, e.g. by in situ hybridization (e.g., according to the examples, below) or it may be isolated from other cell components by common methods known to those skilled in the art before contacting with a probe. Detection methods include Northern blot analysis, RNase protection, in situ methods, e.g. in situ hybridization, in vitro amplification methods (PCR, LCR, QRNA replicase or RNA-transcription/amplification (TAS, 3SR), reverse dot blot disclosed in EP-B1 O 237 362)), immunoassays, Western blot and other detection assays that are known to those skilled in the art.

Products obtained by in vitro amplification can be detected according to established methods, e.g. by separating the products on agarose gels and by subsequent staining with ethidium bromide. Alternatively, the amplified products can be detected by using labeled primers for amplification or labeled dNTPs.

The probes can be detectable labeled, for example, with a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.

Expression of Trp8a, Trp8b, Trp10a and Trp10b, respectively, in tissues can be studied with classical immunohistological methods (Jalkanen et al., J. Cell. Biol. 101 (1985), 976-985; Jalkanen et al., J. Cell. Biol. 105 (1987), 3087-3096; Sobol et al. Clin. Immunopathol. 24 (1982), 139-144; Sobol et al., Cancer 65 (1985), 2005-2010). Other antibody based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{112}In), and technetium rhodamine, and biotin. In addition to assaying Trp8a, Trp8b, Trp 10a or Trp10b levels in a biological sample, the protein can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma. A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I , ^{112}In , ^{99}mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99}mTc . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In

vivo tumor imaging is described in S.W. Burchiel et al., „Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments“. (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B.A. Rhodes, eds., Masson Publishing Inc. (1982)).

The marker Trp8a and Trp8b is also useful for prognosis, for monitoring the progression of the tumor and the diagnostic evaluation of the degree of malignancy of a prostate tumor (grading and staging), e.g. by using in situ hybridization: In a primary carcinoma Trp8 is expressed in about 2 to 10% of carcinoma cells, in a rezidive carcinoma in about 10 to 60% of cells and in metastases in about 60 to 90% of cells.

The present invention also relates to a method for diagnosing endometrial cancer (cancer of the uterus) which comprises contacting a target sample suspected to contain the protein Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA with a reagent which reacts with Trp8a and/or Trp8b or the encoding mRNA and detecting Trp8a and/or Trp8b encoding mRNA. As regards particular embodiments of this method reference is made to the particular embodiments of the method of diagnosing a prostate cancer outlined above.

For evaluating whether the concentration of Trp8a, Trp8b, Trp10a or Trp10b or the concentration of Trp8a, Trp8b, Trp10a or Trp10b encoding mRNA is normal or increased, thus indicative for the presence of a malignant tumor, the measured concentration is compared with the concentration in a normal tissue, preferably by using the ratio of Trp8a:Trp9, Trp8b:Trp9 or Trp10(a or b)/Trp9 for quantification.

Since the prostate carcinoma forms its own basement membrane when growing invasively, it can be concluded that only cells expressing Trp8 and Trp10 are involved in this phenomenon. Thus, it can be concluded that by inhibiting the expression and/or activity of these proteins an effective therapy of cancers like PCA is provided.

Thus, the present invention also relates to a pharmaceutical composition containing a reagent which decreases or inhibits Trp8a, Trp8b, Trp10a and/or Trp10b expression or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b, and a method for preventing, treating, or ameliorating a prostate tumor, endometrial cancer (uterine carcinoma) tumor, a chorion carcinoma, cancer of the lung or melanoma, which comprises administering to a mammalian subject a

therapeutically effective amount of a reagent which decreases or inhibits Trp8a, Trp8b, Trp10a and/or Trp10b expression or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b. Examples of such reagents are the above described antisense RNAs, ribozymes or inhibitors, e.g. specific antibodies. Furthermore, peptides, which inhibit or modulate the biological function of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b may be useful as therapeutical reagents. For example, these peptides can be obtained by screening combinatorial phage display libraries (Cosmix, Braunschweig, Germany) as described by Rottgen, P. and Collins, J. (Gene (1995) 164 (2): 243-250). Furthermore, antigenic epitopes of the Trp8 and Trp10 proteins can be identified by the expression of recombinant Trp8 and Trp10 epitope libraries in *E. coli* (Marquart, A. & Flockerzi, V., FEBS Lett. 407 (1997), 137-140; Trost, C., et al., FEBS Lett. 451 (1999) 257-263 and the consecutive screening of these libraries with serum of patients with cancer of the prostate or of the endometrium. Those Trp8 and Trp10 epitopes which are immunogenic and which lead to the formation of antibodies in the serum of the patients can be then be used as Trp8 or Trp10 derived peptide vaccines for immune interventions against cancer cells which express Trp8 or Trp10. Alternatively to the *E. coli* expression system, Trp8 or Trp10 or epitopes of Trp8 and Trp10 can be expressed in mammalian cell lines such as human embryonic kidney (Hek 293) cells (American Type Culture Collection, ATCC CRL 1573).

Finally, compounds useful for therapy of the above described diseases comprise compounds which act as antagonists or agonists on the ion channels Trp8, Trp9 and Trp10. It could be shown that Trp8 is a highly calcium selective ion channel which in the presence of monovalent (namely sodium) and divalent ions (namely calcium) is only permeable for calcium ions (see Example 4, below, and Figures 3A, C, E). Under physiological conditions, Trp8 is a calcium selective channel exhibiting large inward currents. This very large conductance of Trp8 channels (as wells as Trp9 and Trp10a/b channels) is useful to establish systems for screening pharmacological compounds interacting with Trp-channels including high throughput screening systems. Useful high throughput screening systems are well known to the person skilled in the art and include, e.g., the use of cell lines stably or transiently transfected with DNA sequences encoding Trp8, Trp9 and Trp10 channels in assays to detect calcium signaling in biological systems. Such systems include assays based on Ca-sensitive dyes such as aequorin, apoequorin, Fura-2, Fluo-3 and Indo-1.

Accordingly, the present invention also relates to a method for identifying compounds which act as agonists or antagonists on the ion channels Trp8, Trp9 and/or Trp10, said method comprising contacting a test compound with the ion channel Trp8, Trp9 and/or Trp10, preferably by using a system based on cells stably or transiently transfected with DNA sequences encoding Trp8, Trp9 and/or Trp10, and determining whether said test compound affects the calcium uptake.

For administration the above described reagents are preferably combined with suitable pharmaceutical carriers. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Such carriers can be formulated by conventional methods and can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g. by intravenous, intraperitoneal, subcutaneous, intramuscular, topical or intradermal administration. The route of administration, of course, depends on the nature of the tumor and the kind of compound contained in the pharmaceutical composition. The dosage regimen will be determined by the attending physician and other clinical factors. As is well known in the medical arts, dosages for any one patient depends on many factors, including the patient's size, body surface area, age, sex, the particular compound to be administered, time and route of administration, the kind and stage of the tumor, general health and other drugs being administered concurrently.

The delivery of the antisense RNAs or ribozymes of the invention can be achieved by direct application or, preferably, by using a recombinant expression vector such as a chimeric virus containing these compounds or a colloidal dispersion system. By delivering these nucleic acids to the desired target, the intracellular expression of Trp8a, Trp8b, Trp10a and/or Trp10b and, thus, the level of Trp8a, Trp8b, Trp10a and/or Trp10b can be decreased resulting in the inhibition of the negative effects of Trp8a, Trp8b, Trp10a and/or Trp10b, e.g. as regards the metastasis formation of PCA.

Direct application to the target site can be performed, e.g., by ballistic delivery, as a colloidal dispersion system or by catheter to a site in artery. The colloidal dispersion systems which can be used for delivery of the above nucleic acids include macromolecule complexes, nanocapsules, microspheres, beads and lipid-based systems including oil-in-water emulsions

(mixed), micelles, liposomes and lipoplexes, The preferred colloidal system is a liposome. The composition of the liposome is usually a combination of phospholipids and steroids, especially cholesterol. The skilled person is in a position to select such liposomes which are suitable for the delivery of the desired nucleic acid molecule. Organ-specific or cell-specific liposomes can be used in order to achieve delivery only to the desired tumor. The targeting of liposomes can be carried out by the person skilled in the art by applying commonly known methods. This targeting includes passive targeting (utilizing the natural tendency of the liposomes to distribute to cells of the RES in organs which contain sinusoidal capillaries) or active targeting (for example by coupling the liposome to a specific ligand, e.g., an antibody, a receptor, sugar, glycolipid, protein etc., by well known methods). In the present invention monoclonal antibodies are preferably used to target liposomes to specific tumors via specific cell-surface ligands.

Preferred recombinant vectors useful for gene therapy are viral vectors, e.g. adenovirus, herpes virus, vaccinia, or, more preferably, an RNA virus such as a Retrovirus. Even more preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of such retroviral vectors which can be used in the present invention are: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV) and Rous sarcoma virus (RSV). Most preferably, a non-human primate retroviral vector is employed, such as the gibbon ape leukemia virus (GaLV), providing a broader host range compared to murine vectors. Since recombinant retroviruses are defective, assistance is required in order to produce infectious particles. Such assistance can be provided, e.g., by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. Suitable helper cell lines are well known to those skilled in the art. Said vectors can additionally contain a gene encoding a selectable marker so that the transduced cells can be identified. Moreover, the retroviral vectors can be modified in such a way that they become target specific. This can be achieved, e.g., by inserting a polynucleotide encoding a sugar, a glycolipid, or a protein, preferably an antibody. Those skilled in the art know additional methods for generating target specific vectors. Further suitable vectors and methods for in vitro- or in vivo-gene therapy are described in the literature and are known to the persons skilled in the art; see, e.g., WO 94/29469 or WO 97/00957.

In order to achieve expression only in the target organ, i.e. tumor to be treated, the nucleic acids encoding, e.g. an antisense RNA or ribozyme can also be operably linked to a tissue specific promoter and used for gene therapy. Such promoters are well known to those skilled in the art (see e.g. Zimmermann et al., (1994) *Neuron* 12, 11-24; Vidal et al.; (1990) *EMBO J.* 9, 833-840; Mayford et al., (1995), *Cell* 81, 891-904; Pinkert et al., (1987) *Genes & Dev.* 1, 268-76).

For use in the diagnostic research discussed above, kits are also provided by the present invention. Such kits are useful for the detection of a target cellular component, which is Trp8a, Trp8b, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts, wherein the presence or an increased concentration of Trp8a, Trp8b, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts is indicative for a prostate tumor, endometrial cancer, melanoma, chorion carcinoma or cancer of the lung, said kit comprising a probe for detection of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts. The probe can be detectably labeled. Such probe may be a specific antibody or specific oligonucleotide. In a preferred embodiment, said kit contains an anti-Trp8a-, anti-Trp8b-, anti-Trp9-, anti-Trp10a-and/or anti-Trp10b-antibody and allows said diagnosis, e.g., by ELISA and contains the antibody bound to a solid support, for example, a polystyrene microtiter dish or nitrocellulose paper, using techniques known in the art. Alternatively, said kits are based on a RIA and contain said antibody marked with a radioactive isotope. In a preferred embodiment of the kit of the invention the antibody is labeled with enzymes, fluorescent compounds, luminescent compounds, ferromagnetic probes or radioactive compounds. The kit of the invention may comprise one or more containers filled with, for example, one or more probes of the invention. Associated with container (s) of the kit can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

EXAMPLES

The following Examples are intended to illustrate, but not to limit the invention. While such Examples are typical of those that might be used, other methods known to those skilled in the art may alternatively be utilized.

Example 1: Materials and Methods

(A) Isolation of cDNA clones and Northern blot analysis

Total RNA was isolated from human placenta and prostate using standard techniques. Isolation of mRNA was performed with poly (A)⁺RNA - spin columns (New England Biolabs, Beverly, USA) according to the instructions of the manufacturer. Poly (a)⁺RNA was reverse transcribed using the cDNA choice system (Gibco-BRL, Rockville, USA) and subcloned in λ -Zap phages (Stratagene, La Jolla, USA). An human expressed sequence tag (GenBank accession number 1404042) was used to screen an oligo d(T) primed human placenta cDNA library. Several cDNA clones were identified and isolated. Additional cDNA clones were isolated from two specifically primed cDNA libraries using primers 5'-gca tag gaa ggg aca ggt gg-3' and 5'-gag agt cga ggt cag tgg tcc-3'.

cDNA clones were sequenced using a thermocycler (PE Applied Biosystems, USA) and Thermo Sequenase (Amersham Pharmacia Biotech Europe, Freiburg, Germany). DNA sequences were analyzed with an automated sequencer (Licor, Lincoln, USA).

For Northern blot analysis 5 μ g human poly (A)⁺ RNA from human placenta or prostate were separated by electrophoresis on 0.8 % agarose gels. Poly (A)⁺ RNA was transferred to Hybond N nylon membranes (Amersham Pharmacia Biotech Europe, Freiburg, Germany). The membranes were hybridized in the presence of 50 % formamide at 42°C over night. DNA probes were labelled using [α^{32} P]dCTP and the „ready prime,, labelling kit (Amersham Pharmacia Biotech Europe, Freiburg, Germany). Commercial Northern blots were hybridized according to the distributors instructions (Clontech, Palo Alto, USA).

(B) Construction of expression plasmids and transfection of HEK 293 cells

Lipofections were carried out with the recombinant dicistronic eucaryotic expression plasmid pdiTRP8 containing the cDNA of Trp8b under the control of the chicken β -actin promoter followed by an internal ribosome entry site (IRES) and the cDNA of the green fluorescent protein (GFP). To obtain pdiTRP8 carrying the entire protein coding regions of TRP8b and

the GFP (Prasher, D.C. et al. (1992), *Gene* 111, 229-233), the 5' and 3'-untranslated sequences of the TRP8b cDNA were removed, the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) *Nucleic Acids Research* 15, 8125-8148) was introduced immediately 5' of the translation initiation codon and the resulting cDNA was subcloned into the pCAGGS vector (Niwa, H., Yamamura, K. and Miyazaki, J (1991), *Gene* 8, 193-199) downstream of the chicken β -actin promoter. The IRES derived from encephalomyocarditis virus (Kim, D.G., Kang, H.M., Jang, S.K. and Shin H.S. (1992) *Mol.Cell.Biol.* 12, 3636-3643) followed by the GFP cDNA containing a Ser65Thr mutation (Heim, R., Cubitt, A.B., Tsien, R.Y. (1995) *Nature* 373, 663-664) was then cloned 3' to the TRP8b cDNA. The IRES sequence allows the simultaneous translation of TRP8b and GFP from one transcript. Thus, transfected cells can be detected unequivocally by the development of green fluorescence.

For monitoring of the intracellular Ca^{2+} concentration human embryonic kidney (HEK 293) cells were cotransfected with the pcDNA3-TRP8b vector and the pcDNA3-GFPvector in a molar ratio of 4 : 1 in the presence of lipofectamine (Quiagen, Hilden, Germany). To obtain pcDNA3-TRP8b the entire protein coding region of TRP8b including the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) *Nucleic Acids Research* 15, 8125-8148) was subcloned into the pcDNA3 vector (Invitrogen, Groningen, Netherlands). Calcium monitoring and patch clamp experiments were carried out two days and one day after transfection, respectively.

(C) Chromosomal localization of the Trp8 gene

The chromosomal localization of the human TRP8 gene was performed using NIGMS somatic hybrid mapping panel No.2 (Coriell Institute, Camden, NJ, USA) previously described (Drwina, H.L., Toji, L.H., Kim, C.H., Greene, A.E., Mulivor, R.A. (1993) *Genomics* 16, 311-314; Dubois, B.L. and Naylor, S.L. (1993) *Genomics* 16, 315-319).

(D) In Vitro Translation, glutathione - sepharose and calmodulin agarose binding assay

N- and C-terminal Trp8-fragments were subcloned into the pGEX-4T2 vector (Amersham Pharmacia Europe, Freiburg, Germany) resulting in glutathione-S-transferase (GST)-Trp8 fusion constructs (Fig. 4). The GST-TRP8-fusion proteins were expressed in *E. coli* BL 21 cells and purified using glutathione - sepharose beads (Amersham Pharmacia Biotech Europe, Freiburg, Germany).

In vitro translation of human Trp8 cDNA and *Xenopus laevis* calmodulin cDNA (Davis, T.N. and Thorner, J. Proc.Natl.Acad.Sci. USA 86, 7909-7913.) was performed in the presence of ^{35}S -methionine using the TNT coupled transcription/translation kit (Promega, Madison, USA). Translation products were purified by gel filtration (Sephadex G50, Amersham Pharmacia Biotech Europe, Freiburg, Germany) and equal amounts of ^{35}S labeled probes were incubated for 2 h with glutathione beads bound to GST - Trp8 or calmodulin - agarose (Calbiochem) in 50 mM Tris-HCl, pH 7.4, 0.1 % Triton X-100, 150 mM NaCl in the presence of 1 mM Ca^{2+} or 2 mM EGTA. After three washes, bound proteins were eluted with SDS sample buffer, fractionated by SDS-PAGE and ^{35}S labeled proteins were detected using a Phosphor Imager (Fujifilm, Tokyo, Japan).

(E) Calcium measurements

The intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) was determined by dual wavelength fura-2 fluorescence ratio measurements (Tsien, R.Y. (1988) Trends Neurosci. 11, 419-424) using a digital imaging system (T.I.L.L. Photonics, Planegg, Germany). HEK cells were grown in minimal essential medium in the presence of 10 % fetal calf serum and cotransfected with the pcDNA3-TRP8b vector and the pCDNA3-GFPvector as described above (B). Transfected cells were detected by development of green fluorescence. The cells were loaded with $4\mu\text{M}$ fura-2/AM (Molecular Probes, Oregon, USA) for one hour. After loading the cells were rinsed 3 times with buffer B1 (10 mM Hepes, 115 mM NaCl, 2 mM MgCl_2 , 5mM KCl, pH 7.4) and the $[\text{Ca}^{2+}]_i$ was calculated from the fluorescence ratios obtained at 340 and 380 nm excitation wavelengths as described (Garcia, D.E., Cavalié, A. and Lux, H.D. (1994) J. Neurosci 14, 545-553).

(F) Electrophysiological recordings

HEK cells were transfected with the eucaryotic expression plasmid pdiTRP8 described in (B) and electrophysiological recordings were carried out one day after transfection. Single cells were voltage clamped in the whole cell mode of the patch clamp technique as described (Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85-100; Philipp, S., Cavalié, A., Freichel, M., Wissenbach, U., Zimmer, S., Trost, C., Marquart, A., Murakami, M. and Flockerzi, V. (1996) EMBO J. 6166-6171). The pipette solution contained (mM): 140 aspartic acid, 10 EGTA, 10 NaCl, 1 MgCl_2 , 10 Hepes (pH 7.2 with CsOH) or 125 CsCl, 10 EGTA, 4 CaCl_2 , 10 Hepes (pH 7.2 with CsOH). The bath solution contained (mM): 100 NaCl, 10 CsCl, 2 MgCl_2 , 50 mannitol, 10 glucose, 20

Hepes (pH 7.4 with CsOH) and 2 CaCl₂, or no added CaCl₂ (-Ca²⁺ solution). Divalent free bath solution contained (mM): 110 N-methyl-D-glucamine (NMDG). Whole cell currents were recorded during 100 msec voltage ramps from -100 to +100 mV at varying holding potentials.

(G) In Situ Hybridization

In situ hybridizations were carried out using formalin fixed tissue slices of 6 - 8 µm thickness. The slices were hydrated and incubated in the presence of PBS buffer including 10 µg / ml proteinase K (Roche Diagnostics, Mannheim, Germany) for 0.5 h. The slices were hybridized at 37°C using biotinylated deoxy-oligonucleotides (0.5 pmol / µl) in the presence of 33 % formamide for 12 h. Furthermore the slices were several times rinsed with 2 x SSC and incubated at 25°C for 0.5 h with avidin / biotinylated horse raddish peroxidase complex (ABC, DAKO, Santa Barbara, USA). After several washes with PBS buffer the slices were incubated in the presence of biotinylated tyramid and peroxide (0.15 % w/v) for 10 min, rinsed with PBS buffer and additionally incubated with ABC complex for 0.5 h. The slices were washed with PBS buffer and incubated in the presence of DAB solution (diaminobenzidine (50µg / ml), 50 mM Tris/EDTA buffer pH 8.4, 0.15 % H₂O₂ in N,N - dimethyl-formamide; Merck, Darmstadt, Germany), The detection was stopped after 4 minutes by incubating the slides in water. Tyramid was biotinylated by incubating NHS-LC Biotin (sulfosuccinimidyl-6-(biotinimid)-hexanoat), 2.5 mg / ml; Pierce, Rockford, USA) and tyramin-HCl (0.75 mg / ml, Sigma) in 25 mM borate buffer pH 8.5 for 12 h. The tyramid solution was diluted 1 - 5 : 1000 in PBS buffer.

(H) GenBank accession numbers: TRP8a, Aj243500; TRP8b Aj243501

Example 2: Expression of TRP8 transcripts

In search of proteins distantly related to the TRP family of ion channels, an human expressed sequence tag (EST, GenBank accession number 1404042) was identified in the GenBank database using BLAST programmes (at the National Center for Biotechnology Information (NCBI); Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J.J. (1990) Mol. Biol. 5, 403-410) being slightly homologous to the VR1 gene. Several human placenta cDNA libraries were constructed and screened with this EST DNA as probe. Several full length

cDNA clones were identified and isolated. The full length cDNA clones encoded two putative proteins differing in three amino acids and were termed Trp8a and Trp8b (Fig. 1c, 2a, 7 and 8A). This finding was reproduced by isolating cDNA clones from two cDNA libraries constructed from two individual placentas. The derived protein sequence(s) comprises six transmembrane domains, a characteristic overall feature of trp channels and related proteins (Fig.: 1b). The sequence is closely related to the meanwhile published calcium uptake transport protein 1 (CaT1), isolated from rat intestine (Peng, J.B., Chen, X.Z., Berger, U.V., Vassilev, P.M., Tsukaguchi, H., Brown, E.M. and Hediger M.A.(1999) J Biol Chem. 6;274, 22739-22746) and to the epithelial calcium uptake channel (ECaC) isolated from rabbit kidney (Hoenderop, J.G., van der Kemp, A.W., Hartog, A., van de Graaf, S.F., van Os, C.H., Willems, P.H. and Bindels, R.J. (1999) J Biol Chem. 26;274, 8375-8378). Expression of Trp8a/b transcripts are detectable in human placenta, pancreas and prostate (Fig.: 5) and the size of the Northern signal (3.0 kb) corresponds with the size of the isolated full length cDNAs. In addition, a shorter transcript of 1.8 kb, probably a splice variant, is detectable in human testis. The Trp8 mRNA is not expressed in small intestine or colon (Fig.: 5) implicating that Trp8 is not the human ortholog of the rat CaT1 or rabbit ECaC proteins. To investigate whether there are other related sequences Trp8a/b derived primers (UW241, 5'-TAT GAG GGT TCA GAC TGC-3' and UW242, 5'-CAA AGT AGA TGA GGT TGC-3') were used to amplify a 105 bp fragment from human genomic DNA being 95% identical on the nucleotide level to the Trp8 sequence (data not shown). This indicates the existence of several similar sequences in humans at least at the genomic level.

Example 3: Two variants of the Trp8 protein (Trp8a and Trp8b) arise by polymorphism

Two variants of the Trp8 cDNA were isolated from human placenta (Fig.: 2A, 7 and 8A) which encoded two proteins which differ in three amino acids and were termed Trp8a and Trp8b. Trp8a/b specific primers were designed to amplify a DNA fragment of 458 bp of the Trp8 gene from genomic DNA isolated from human T-lymphocytes (primer pair: UW243, 5'-CAC CAT GTG CTG CAT CTA CC-3' and UW244, 5'-CAA TGA CAG TCA CCA GCT CC-3'). The amplification product contains a part of the sequence where the derived protein sequence of Trp8a comprises the amino acid valine and the Trp8b sequence methionine as well as a silent base pair exchange (g versus a) and an intron of 303bp (Fig.: 2.A, B). Both variants of the Trp8 genes (a,b) were amplified from genomic DNA in equal amounts indicating the existence of both variants in the human genome and therefore being not the

result of RNA editing (Fig.: 2B). The Trp8a gene can be distinguished from the Trp8b gene by cutting the genomic fragment of 458bp with the restriction enzyme Bsp1286I (Fig. 2B). Using human genomic DNA isolated from blood of twelve human subjects as template, the 458bp fragment was amplified and restricted with BSP1286I. In eleven of the tested subjects only the Trp8b gene is detectable, while one subject (7) contains Trp8a and Trp8b genes (Fig.: 2D). These implicates that the two Trp8 variants arise by polymorphism and do not represent individual genes. Using Trp8 specific primers and chromosomal DNA as template, the Trp8 locus is detectable on chromosome 7 (Fig.: 2C).

Example 4: Trp8b is a calcium permeable channel

The protein coding sequence of the Trp8b cDNA was subcloned into pcDNA3 vector (Invitrogen, Groningen, Netherlands) under the control of the cytomegalovirus promotor (CMV). Human embryonic kidney (HEK 293) cells were cotransfected with the Trp8b pcDNA3 construct (pcDNA3-Trp8b vector) and the pcDNA3-GFPvector encoding the green fluorescent protein (GFP) in 4:1 ratio. The Trp8b cDNA and the cDNA of the reporter, GFP, was transiently expressed in human embryonic kidney (HEK 293) cells. The intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and changes of $[\text{Ca}^{2+}]_i$ were determined by dual wavelength fura-2 fluorescence ratio measurements (Fig.: 3F) in cotransfected cells which were identified by the green fluorescence of the reporter gene GFP.

Dual wavelength fura-2 fluorescence ratio measurement is a standard procedure (e.g. in: An introduction of Molecular Neurobiology (ed. Hall, Z.W.) Sinauer Associates, Sunderland, USA (1992)) using fura-2, which is a fluorescent Ca^{2+} sensitive dye and which was designed by R.Y.Tsien (e.g. Trends Neurosci. 11, 419-424 (1988) based upon the structure of EGTA. Its fluorescence emission spectrum is altered by binding to Ca^{2+} in the physiological concentration range. In the absence of Ca^{2+} , fura-2 fluoresces most strongly at an excitation wavelength of 385 nm; when it binds Ca^{2+} , the most effective excitation wavelength shifts to 345 nm. This property is used to measure local Ca^{2+} concentrations within cells. Cells can be loaded with fura-2 esters (e.g. fura-2AM) that diffuse across cell membranes and are hydrolyzed to active fura-2 by cytosolic esterases.

In the presence of 1mM Ca^{2+} , Trp8 expressing cells typically contained more than 300 nM cytosolic Ca^{2+} , while non transfected controls contained less than 100 nM Ca^{2+} ions (Fig. 3F).

When Trp8b transfected cells were incubated without extracellular Ca^{2+} , the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) decreased to levels comparable to non transfected cells. Readdition of 1mM Ca^{2+} to the bath resulted in significant increase of the cytosolic $[\text{Ca}^{2+}]$ in Trp8b transfected cells, but not in controls (Fig.: 3F). After readdition of Ca^{2+} ions to the bath solution, the cytosolic Ca^{2+} concentration remains on a high steady state level in the Trp8b transfected cells.

Example 5: Trp8 expressing cells show calcium selective inward currents

To characterize in detail the electrophysiological properties of TRP8, TRP8 and GFP were coexpressed in HEK293 cells using the dicistronic expression vector pdiTRP8 and measured currents using the patch clamp technique in the whole cell mode (Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) *Pflugers Arch.*, 391, 85-100).

The eucaryotic expression plasmid pdiTRP8 contains the cDNA of Trp8b under the control of the chicken β -actin promotor followed by an internal ribosome entry side (IRES) and the cDNA of the green fluorescent protein (GFP). To obtain pdiTRP8 carrying the entire protein coding regions of TRP8b and the GFP (Prasher, D.C. et al. (1992), *Gene* 111, 229-233), the 5' and 3'-untranslated sequences of the TRP8b cDNA were removed, the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) *Nucleic Acids Research* 15, 8125-8148) was introduced immediately 5' of the translation initiation codon and the resulting cDNA was subcloned into the pCAGGS vector (Niwa, H., Yamamura, K. and Miyazaki, J. (1991), *Gene* 8, 193-199) downstream of the chicken β -actin promotor. The IRES derived from encephalomyocarditis virus (Kim, D.G., Kang, H.M., Jang, S.K. and Shin H.S. (1992) *Mol.Cell.Biol.* 12, 3636-3643) followed by the GFP cDNA containing a Ser65Thr mutation (Heim, R., Cubitt, A.B., Tsien, R.Y. (1995) *Nature* 373, 663-664) was then cloned 3' to the TRP8b cDNA. The IRES sequence allows the simultaneous translation of TRP8b and GFP from one transcript. Thus, transfected cells can be detected unequivocally by the development of green fluorescence.

In the presence of 2 mM external calcium, Trp8b transfected HEK cells show inwardly rectifying currents, the size of which depends on the level of intracellular calcium and the electrochemical driving force. The resting membrane potential was held either at -40 mV, or, to lower the driving force for calcium influx in between pulses, at + 70 mV. Current traces

were recorded in response to voltage ramps from -100 to $+100$ mV, that were applied every second. To monitor inward and outward currents over time, we analyzed the current size at -80 and $+80$ mV of the ramps. Figure 3A shows a representative trace of the current at -80 mV over time. Both at a holding potential of -40 mV or at $+70$ mV, the currents are significantly larger than in cells transfected with only the GFP containing vector (Fig.: 3E). Interestingly, after changing to a positive holding potential, current size in Trp8 transfected cells slowly increases and reaches steady state after approximately 70 seconds (Fig.: 3A). To determine the selectivity of the induced currents, we then perfused the cells with solutions that either contain no sodium, no added Ca^{2+} (Fig. 3A, C) or a sodium containing, but divalent ion free bath solution. To control for the effect of the solution change alone, we also perfused with normal bath (see puff in Fig. 3A). While removal of external Ca^{2+} completely abolishes the trp 8 induced currents - the remaining current being identical in size and shape to the control (Fig.: 3A, C, E), removal of external sodium has no effect (Fig.: 3E). An important hallmark of calcium selective channels (e.g. Vennekens, R., Hoenderop, G.J., Prenen, J., Stuiover, M., Willems, PHGM, Droogmans, G., Nilius, B. and Bindels, R.J.M (1999) *J. Biol. Chem.* 275, 3963-3969), is their ability to conduct sodium only if all external divalent ions, namely Ca^{2+} and magnesium are removed. To test whether the trp 8 channel conforms with this phenomenon normal bath solution was switched to a solution containing only sodium and 1 mM EGTA. As can be seen in Figure 3B and D, Trp8 transfected cells can now conduct very large sodium currents. Interestingly, immediately after the solution change, the currents first become smaller before increasing rapidly, indicating that the pore may initially still be blocked by calcium a phenomenon usually called anomalous mole fraction behaviour (Warnat, J., Philipp, S., Zimmer, S., Flockerzi, V., and Cavalié A. (1999) *J. Physiol. (Lond)* 518, 631-638). The measured outward currents of Trp8 transfected cells in normal bath solution are not significantly different from non-transfected control cells or cells which only express the reporter gene GFP. As the removal of external Ca^{2+} abolishes the Trp8 specific current, the remaining current was subtracted from the current before the solution change to obtain the uncontaminated Trp8 conductance (see inset in Fig.: 3C). As expected from the given ionic conditions (high EGTA inside, 2 mM Ca^{2+} outside), the current-voltage relationship now shows prominent inward rectification with little to no outward current.

Both the time course of the development of Trp8 currents and the size of the currents depend on the frequency of stimulation (data not shown), the internal and external Ca^{2+} concentration

and the resting membrane potential, suggesting that Trp8 calcium conductance is intricately regulated by a Ca^{2+} mediated feedback mechanisms.

Example 6: Ca^{2+} / calmodulin binds to the C-terminus of the Trp8 protein

To test whether calmodulin, a prime mediator of calcium regulated feedback, is involved, first it was investigated biochemically whether Trp8 protein can bind calmodulin. Trp8 cDNA was in vitro translated in the presence of ^{35}S -methionine and the product incubated with calmodulin-agarose beads. After several washes either in the presence or absence of Ca^{2+} , the beads were incubated in Laemmli buffer and subjected to SDS-polyacrylamide gel electrophoresis. In the presence of Ca^{2+} (1mM), but not in the absence of Ca^{2+} , Trp8 protein binds to calmodulin (Fig.: 4B).

To narrow down the binding site, two approaches were undertaken: Firstly, GST-TRP8 fusion proteins of various intracellular domains of Trp8 were constructed, expressed in *E. coli* and bound to glutathione sepharose beads. These beads were then incubated with in vitro translated ^{35}S - labeled calmodulin, washed and subjected to gel electrophoresis. Secondly, truncated versions of in vitro translated Trp8 protein were used in the above described binding to calmodulin-agarose. As shown in Figure 4A, and C, fusion proteins of the N-terminal region (N1, N2) of Trp8 did not bind calmodulin, while C-terminal fragments (C1, C2, C3, C4) showed calmodulin binding in the presence of calcium (for localization of fragments within the entire Trp8 protein see Fig. 4C). Accordingly, a truncated version of in vitro translated Trp8, which lacks the C-terminal 32 amino acid residues did not bind to calmodulin-agarose (4B). We have restricted the calmodulin binding site to amino acid residues 691 to 711 of the Trp8 protein. This calmodulin binding site does not resemble the typical conserved IQ - motif of conventional myosins, but has limited sequence homology to the calcium dependent calmodulin binding site 1 of the transient receptor potential like (trpl) protein of *Drosophila melanogaster* (Warr and Kelly, 1996) with several charged amino acid residues conserved. The sequence of the calmodulin binding site of the Trp8 protein resembles a putative amphipathic α -helical wheel structure with a charged and a hydrophobic site according to a model proposed by Erickson-Vitanen and De Grado (1987, *Methods Enzymol.* 139, 455-478.).

Example 7: Expression of Trp8 transcripts in human placenta and pancreas

Several slides from a human placenta of a ten week old abort were used for in situ hybridization experiments. The in situ hybridization experiments revealed expression of Trp8 transcripts in human placenta (Fig.: 5B). Expression was detectable in trophoblasts and syncytiotrophoblasts of the placenta, but not in Langhans cells.

Trp8 transcripts are detectable in human pancreas (Fig.: 5A). Therefore Trp8 probes were hybridized to tissue sections of human pancreas. The pancreatic tissues were removed from patients with pancreas cancer. Trp8 expression is detectable in pancreatic acinar cells, but not in Langerhans islets (Fig.: 5C). No Trp8 expression was found in regions of pancreatic carcinomas (data not shown).

Furthermore, the Trp8 cDNA is not detectable in human colon nor in human kidney by in situ hybridization as well as by Northern analysis (Fig.: 5A, D). The Northern results taken together with the in situ expression data indicate that the Trp8 protein is not the human ortholog of the CaT1 and ECaC channels cloned from rat intestine (Peng, J.B., Chen, X.Z., Berger, U.V., Vassilev, P.M., Tsukaguchi, H., Brown, E.M. and Hediger M.A.(1999) J Biol Chem. 6;274, 22739-22746) and from rabbit kidney (Hoenderop, J.G., van der Kemp, A.W., Hartog, A., van de Graaf, S.F., van Os, C.H., Willems, P.H. and Bindels, R.J. (1999) J Biol Chem. 26;274, 8375-8378), respectively. Trp8 is unlikely to represent the human version of CaT1 as its expression is undetectable in the small intestine and colon tissues where CaT1 is abundantly expressed. If, however, Trp8 is the human version of rat CaT1, a second gene product appears to be required for Ca²⁺ uptake in human small intestine and colon attributed to CaT1 in rat small intestine and colon.

Example 8: Differential expression of Trp8 transcripts in benign and malign tissue of the prostate

The Trp8 transcripts are expressed in human prostate as shown by hybridization of a Trp8 probe to a commercial Northern blot (Clontech, Palo Alto, USA) (Fig.: 5A). Trp8 transcripts were not detectable by Northern blot analysis using pooled mRNA of patients with benign prostatic hyperplasia (BPH) (Fig.: 5A, prostate*). To examine Trp8 expression on the cellular

level, sections of prostate tissues were hybridized using Trp8 specific cDNA probes (Table 3). Expression of Trp8 transcripts is not detectable in normal prostate (n = 3), benign hyperplasia (BPH, n = 15) or prostatic intraepithelial neoplasia (PIN, n = 9) (Fig.: 6A, C, E). Trp8 transcripts were only detectable in prostate carcinoma (PCA), although with different expression levels. Low expression levels were found in primary carcinomas (2 - 10 % of the carcinoma cells, n = 8) (Fig.: 7B). Much stronger expression was detectable in rezidive carcinoma (10 - 60 %) (Fig.: 7D, n = 6) and metastases of the prostate (60 - 90 %, n = 4) (Fig.: 7F). Thus it has to be concluded that the commercial Northern blot used in Fig.: 5A contains not only normal prostate mRNA as indicated by the distributor. According to the distributors instructions the prostate mRNA used for this Northern blot was collected from 15 human subjects in the range of 14 to 60 years of age. This prostate tissue was not examined by pathologic means. Since Trp8 expression is not detectable in normal or benign prostate, this finding implicates that the mRNA used for this Northern blot was extracted in part from prostatic carcinoma tissue. To summarize, Trp8 expression is only detectable in malign prostate and, thus, the Trp8 cDNA is a marker for prostate carcinoma. The results are summarized in Table 4.

Table 3

Trp8 probes used for in situ hybridization:

Probes (antisense)

- 1.) 5' TCCGCTGCCGGTTGAGATCTTGCC 3'
- 2.) 5' CTTGCTCCATAGGCAGAGAATTAG 3'
- 3.) 5' ATCCTCAGAGCCCCGGGTGTGGAA3'

Controls (sense)

- 1.) 5' GGCAAGATCTCAACCGGCAGCGGA 3'
- 2.) 5' CTAATTCTCTGCCTATGGAGCAAG 3'
- 3.) 5' TTCCACACCCGGGGCTCTGAGGAT 3'

Table 4

Prostate	total	negative	positive
normal	3	3	0
BPH	15	15	0
PIN	9	9	0

carcinoma

18

1

17

(B) Differential expression of Trp8 transcripts in benign and malign tissue of the uterus

Moreover it could be shown that Trp8 is expressed in endometrial cancer (also called cancer of the uterus, to be distinguished from uterine sarcoma or cancer of the cervix) whereas no expression was observed in normal uterus tissue. Thus, Trp8 also is a specific marker for the diagnosis of the above cancer (Fig. 12).

Example 9: Characterization of Trp9

The complete protein coding sequence of Trp9 was determined (Fig. 9). Trp 9 transcripts are predominantly expressed in the human prostate and in human colon. As it could be shown by Northern blot analysis, there is no difference of the expression of TRP9 in benign prostata hyperplasia (BPH, Fig. 13, upper panel left) or prostate carcinoma (Fig. 13, upper panel right). However, Trp9 is useful as a reference marker for prostata carcinoma, i.e. can be used for quantifying the expression level of Trp8. The ratio of the expression of Trp8:Trp9 in patients and healthy individuals is useful for the development of a quantitative assay.

Example 10: Characterization of Trp10

The complete protein coding sequence of TRP10 (a and b) was determined by biocomputing (Fig. 10 and 11). Using a 235 bp fragment of the Trp10 cDNA as probe in Northern blot analysis TRP10 transcripts could only be detected in mRNA isolated from individuals with prostate cancer (Fig. 13, bottom panel) but not in mRNA isolated from benign tissue of the prostate (prostate BPH) nor in mRNA isolated from heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. The 235 bp cDNA fragment of the Trp10 cDNA was amplified using the primer pair UW248 5'-ACA GCT GCT GGT CTA TTC C-3' and UW249 5'-TAT

GTG CCT TGG TTT GTA CC-3' and prostate cDNA as template. In summary, Trp10a and Trp10b, like TRP8 are also expressed in malignant prostate tissue. So far, its expression could not be observed in any other tissue examined (see above). Thus, Trp 10a and Trp10b are also useful markers which are specific for malignant prostate tissue.

Furthermore, database searches in public databases of the national center for biological information (NCBI) revealed the existence of several expressed sequence tags (EST clones) being in part identical to the Trp10 sequence. These EST clones were originally isolated from cancer tissues of lung, placenta, prostate and from melanoma. These clones include the clones with the following accession numbers: BE274448, BE408880, BE207083, BE791173, AI671853, BE390627. The results demonstrate that cancer cells of these tissues express Trp10 related transcripts whereas no expression of Trp10 transcripts in the corresponding healthy tissues are detectable (Figure 13). Furthermore, it could be shown that in cancer cells of melanoma and prostate cancer Trp10 transcripts are expressed as shown by in situ hybridizations using 4 antisense probes (Figure 14A – E and 13K-O and Table 2, above). Furthermore, it could clearly be shown that cancer cells of these tissues expressing Trp10 transcripts also express Trp10-antisense transcripts as shown in Figure 14F-J, Figure 14P-R and Figure 14T by in situ hybridizations using 4 sense probes (Table 2, above). The in situ hybridization experiments demonstrate that detection of a subset of cancer cells derived from carcinoma of lung, placenta, prostate and melanoma is feasible using antisense as well as sense probes complementary to Trp10 transcripts or complementary to Trp10-antisense transcripts, respectively.

The foregoing is meant to illustrate but not to limit the scope of the invention. The person skilled in the art can readily envision and produce further embodiment, based on the above teachings, without undue experimentation.

What Is claimed Is:

1. An isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b or a protein exhibiting biological properties of Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being selected from the group consisting of
 - (a) a nucleic acid molecule encoding a protein that comprises the amino acid sequence depicted in Figure 7, 8A, 9, 10 or 11;
 - (b) a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9, 10 or 11;
 - (c) a nucleic acid molecule included in DSMZ Deposit No. DSM 13579, DSM 13580, DSM 13584, DSM 13581 or DSM....;
 - (d) a nucleic acid molecule which hybridizes to a nucleic acid molecule specified in (a) to (c);
 - (e) a nucleic acid molecule the nucleic acid sequence of which deviates from the nucleic sequences specified in (a) to (d) due to the degeneration of the genetic code; and
 - (f) a nucleic acid molecule, which represents a fragment, derivative or allelic variation of a nucleic acid sequence specified in (a) to (e).
2. A recombinant vector containing the nucleic acid molecule of claim 1
3. The recombinant vector of claim 2 wherein the nucleic acid molecule is operatively linked to regulatory elements allowing transcription and synthesis of a translatable RNA in prokaryotic and/or eukaryotic host cells.
4. A recombinant host cell which contains the recombinant vector of claim 3.
5. The recombinant host cell of claim 4, which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.
6. An isolated protein exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b which is encoded by a nucleic acid molecule of claim 1.
7. A recombinant host cell that expresses the isolated protein of claim 6.

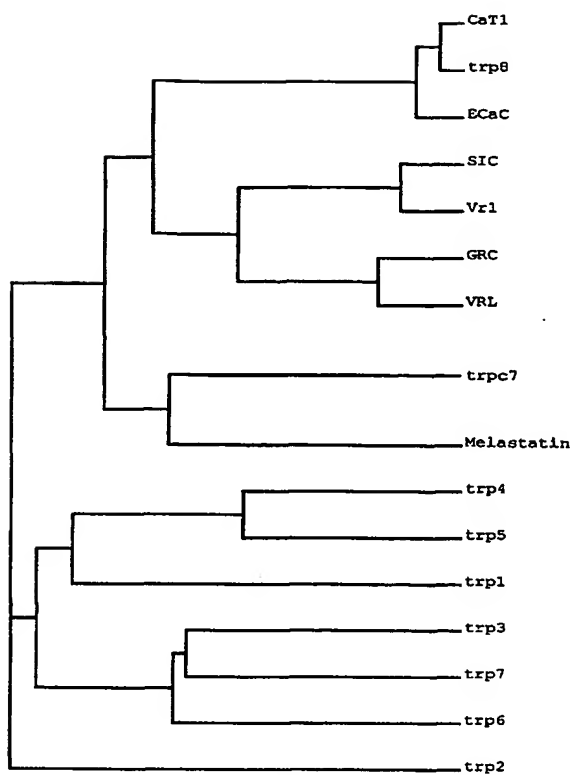
8. A method of making an isolated protein exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b comprising:
(a) culturing the recombinant host cell of claim 6 under conditions such that said protein is expressed; and
(b) recovering said protein.
9. The protein produced by the method of claim 8.
10. An antisense RNA sequence characterized in that it is complementary to an mRNA transcribed from a nucleic acid molecule of claim 1 or a part thereof and can selectively bind to said mRNA or part thereof, said sequence being capable of inhibiting the synthesis of the protein encoded by said nucleic acid molecule.
11. A ribozyme characterized in that it is complementary to an mRNA transcribed from a nucleic acid molecule of claim 1 or a part thereof and can selectively bind to and cleave said mRNA or part thereof, thus inhibiting the synthesis of the protein encoded by said nucleic acid molecule.
12. An inhibitor characterized in that it can suppress the activity of the protein of claim 6.
13. A method for diagnosing a prostate carcinoma which comprises contacting a target sample suspected to contain the protein Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA with a reagent which reacts with Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA and detecting Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA.
14. The method of claim 13, wherein the reagent is a nucleic acid.
15. The method of claim 13, wherein the reagent is an antibody.
16. The method of claim 13, wherein the reagent is detectably labeled.

17. The method of claim 16, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.
18. A method for diagnosing an endometrial cancer (carcinoma of the uterus) which comprises contacting a target sample suspected to contain the protein Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA with a reagent which reacts with Trp8a and/or Trp8b or the Trp8a and/or Trp8a and/or trp8b encoding mRNA and detecting Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA.
19. The method of claim 18, wherein the reagent is a nucleic acid.
20. The method of claim 18, wherein the reagent is an antibody.
21. The method of claim 18, wherein the reagent is detectably labeled.
22. The method of claim 21, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.
23. A method for diagnosing a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense RNA or Trp10a and/or Trp10b related antisense RNA.
24. A method for preventing, treating, or ameliorating a prostate tumor, endometrial cancer (carcinoma of the uterus) tumor, a chorion carcinoma, cancer of the lung or melanoma, which comprises administering to a mammalian subject a therapeutically effective amount of a reagent which decreases or inhibits expression of Trp8a, Trp8b, Trp10a and/or Trp10b and/or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b.
25. The method of claim 24, wherein the reagent is a nucleotide sequence comprising an antisense RNA.

26. The method of claim 24, wherein the reagent is a nucleotide sequence comprising a ribozyme.
27. The method of claim 24, wherein the reagent is an inhibitor of Trp8a, Trp8b, Trp10a and/or Trp10b.
28. The method of claim 27, wherein the reagent is an anti-Trp8a-, anti Trp8b-, anti-Trp10a- and/or anti-Trp10b antibody or a fragment thereof.
29. A diagnostic kit useful for the detection of Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts in a sample, wherein the presence of an increased concentration of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts is indicative for a prostate tumor, endometrial cancer (cancer of the uterus) tumor, a chorion carcinoma, cancer of the lung or melanoma, said kit comprising a probe for detection of Trp8a, Trp8b, Trp9, Trp10a or Trp10b or Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts.
30. The kit of claim 29, wherein the target component to be detected is Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b and the probe is an antibody.
31. A method for identifying a compound which acts as an agonist or antagonist on the ion channels Trp8, Trp9 and/or Trp10, said method comprising contacting a test compound with the ion channel Trp8, Trp9 and/or Trp10, and determining whether said test compound affects the calcium uptake.

Figs. 1A and 1B

A



B

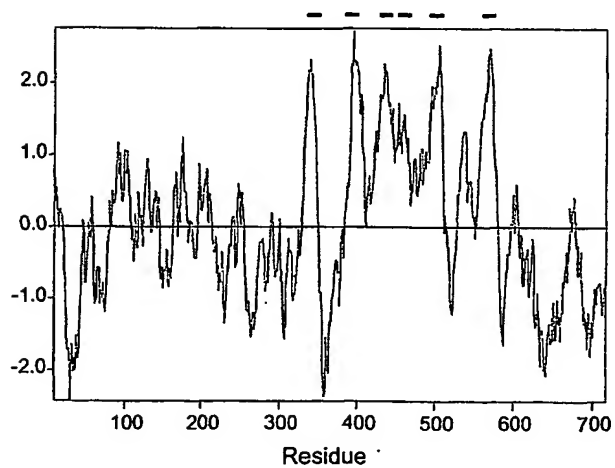
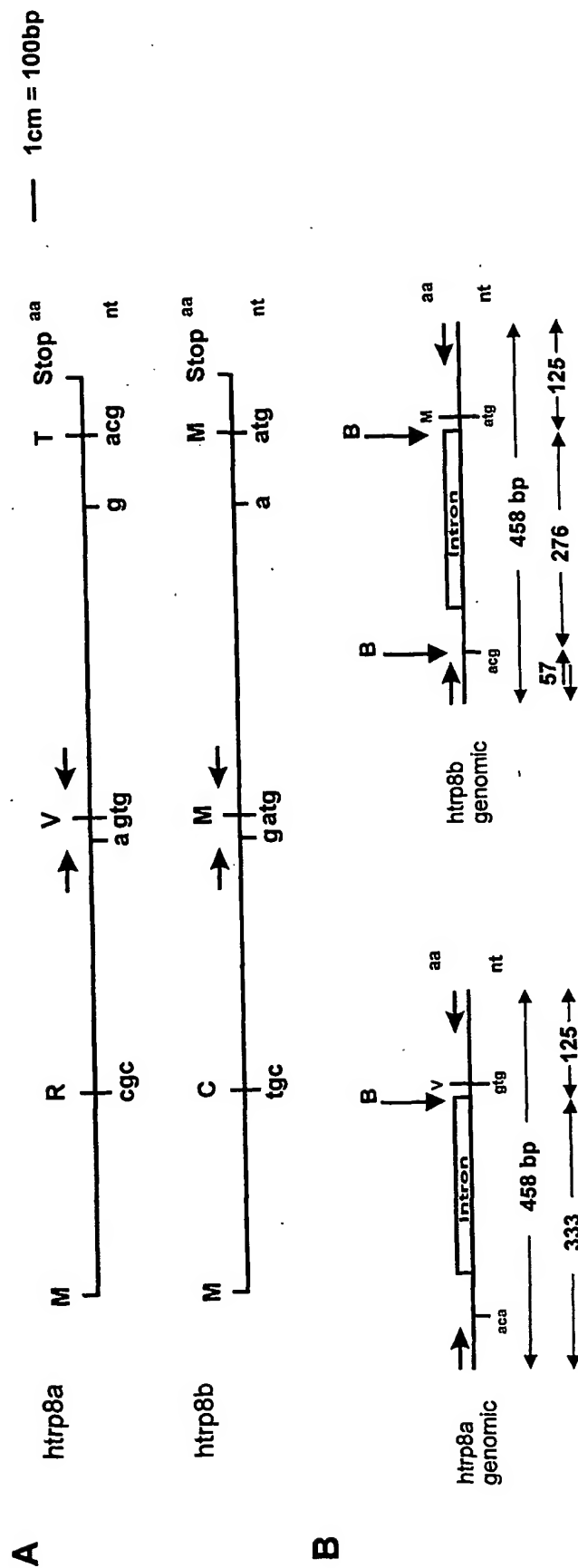


Fig. 1C

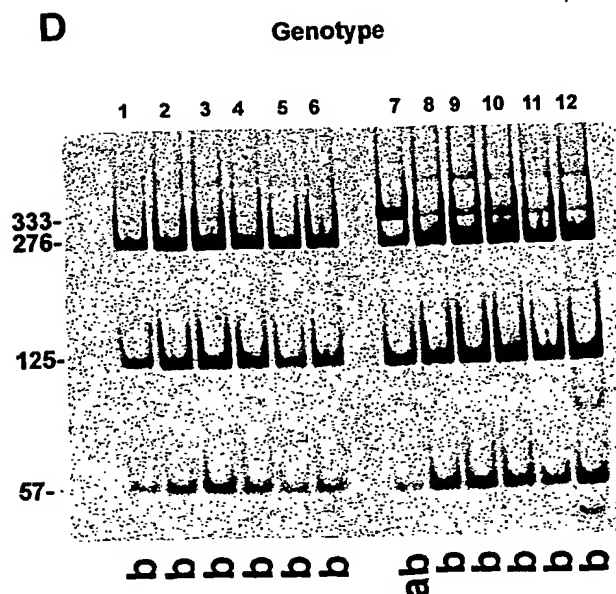
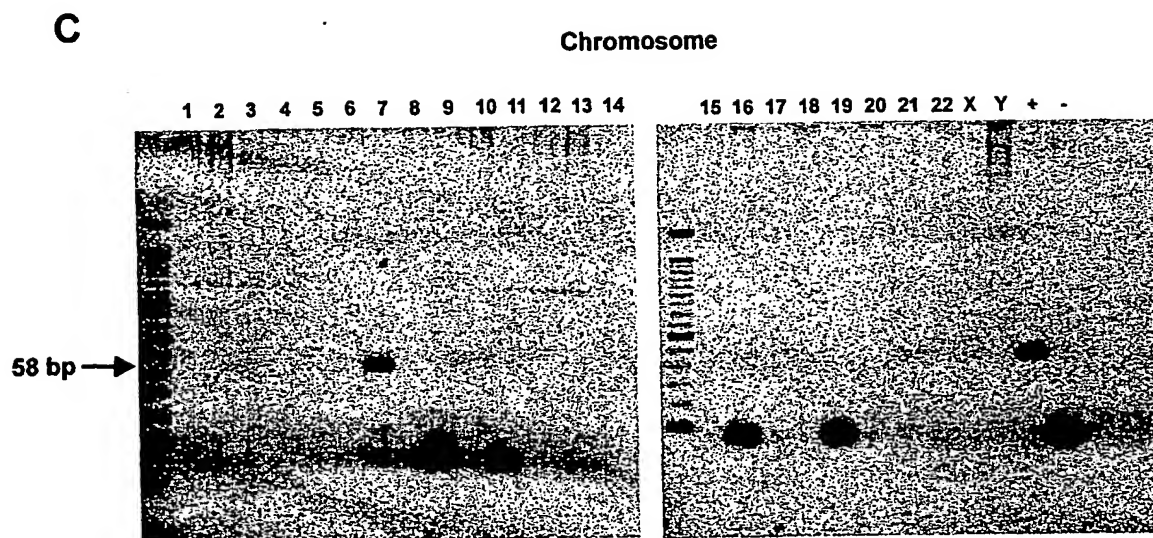
C

htp8A	MDQRASLDSESESPQENSLDPPDRDNCKPPVKKPHIFITRSRTLFGKGESEASP	MG	2
htp8B		MG	2
Vr1			60
ECaC		MG	2
htp8A	LSLEKKG---LILCLMSKFCRWFORR---ESWAGSRNEQNLOOK-RIWESP-LLLR		52
htp8B	LSLEKKG---LILCLMSKFCRWFORR---ESWAGSRNEQNLOOK-RIWESP-LLLR		52
Vr1	LDCEYDEGLASCPITVSSVLTIQPGDGPASVRPSSQDSVAGEKPPALYRRSIFDA		120
ECaC	ACPEKAKS---PWALQKLLISWVGE---QDWQYRVRVNMLOQE-RIRGSP-LLOQ		52
htp8A	AKNDVQANLNGZAYEDCKVH---ORGAVGSAHHRAN-YDN-LEAAMVMEAR		102
htp8B	AKNDVQANLNGZAYEDCKVH---ORGAVGSAHHRAN-YDN-LEAAMVMEAR		102
Vr1	VAGSNQCPESLPFLQSRKRLTDFSEFKDPETGKCYLFAKMNLRGQNDTIALALDVA		180
ECaC	AKNDLRLKRLILNQSDFQ---ORGAVGSAHHRAN-YDN-LEAATLMEAN		102
htp8A	-----PELVFEMTSELVEGQALHTVVMKQVRAILARRASUSRRATTAERRSP		156
htp8B	-----PELVFEMTSELVEGQALHTVVMKQVRAILARRASUSRRATTAERRSP		156
Vr1	RKYSLSKQFVNASYTDSYKQALHTAERRATTAATLVENGADQRAKQDFKQTK		240
ECaC	-----PELVFAPLCEPFVQALHTVVMKQVRAILARRASUSRRATTAERRSP		156
htp8A	INLIYFERRPLSFAAGVNSEETVRLIEHG---ADIRBQDSLENTVHLILQF---		207
htp8B	INLIYFERRPLSFAAGVNSEETVRLIEHG---ADIRBQDSLENTVHLILQF---		207
Vr1	GRPGFTRERPLSLAAGTNQLATVKFLQNSWQFQHSRRPSQENTVHLILVEADNTVD		300
ECaC	INLIYFERRPLSFAAGVNSEETVRLIEHG---ADIRBQDSLENTVHLILQF---		207
htp8A	SKTEACQHTLILSYDRGHQLOPDLNVRHQSLETFKLGVEENTVMEQHLQ---		261
htp8B	SKTEACQHTLILSYDRGHQLOPDLNVRHQSLETFKLGVEENTVMEQHLQ---		261
Vr1	SKTEVTSHTLILGAKLPTLKEETTRKSLTELALASSKGLVAYILGREIHEP		360
ECaC	SKTEACQHTLILSYDRGHQLOPDLNVRHQSLETFKLGVEENTVMEQHLQ---		261
htp8A	-----KRHTQNTYRELSTLYNTELESSGDEQLELLIITTK-KREAR-QIHDQTEVK		314
htp8B	-----KRHTQNTYRELSTLYNTELESSGDEQLELLIITTK-KREAR-QIHDQTEVK		314
Vr1	ECRLSRPTETAVGRVSSLSVSCSLVTC-EDSVIEVNAVSSSETENHMLAVEELN		420
ECaC	-----KRHTQNTYRELSTLYNTELESSGDEQLELLIITTK-KREAR-QIHDQTEVK		314
htp8A	ELVSLKRYGRPYECMLGAILITICCHMCCTIRLAPRTNNRTSRRNTLLQQLQ		374
htp8B	ELVSLKRYGRPYECMLGAILITICCHMCCTIRLAPRTNNRTSRRNTLLQQLQ		374
Vr1	RILQDQKREVRKRYEYFNFVQCMQIIPARAYEEVEG---LPP---YKLRN		468
ECaC	ELVSLKRYGRPYECVLASLILICCTOCIRLKLRODNRTOEDITLQQLQ		374
htp8A	-----S1-----		
htp8B	EAYTPKDDILVGLVNTIGAILILLVEVPDIFRMGVTRFFGQTILGGPFRVLIITYAF		434
htp8B	EAYTPKDDILVGLVNTIGAILILLVEVPDIFRMGVTRFFGQTILGGPFRVLIITYAF		434
Vr1	-----FYGVYFVTEILSYSGVYFFRFGIQTFLQRPQ---LKSLEVDYSIELFVQSL		522
ECaC	EAYTPKDDILVGLVNTIGAILILLVEVPDIFRMGVTRFFGQTILGGPFRVLIITYAS		434
htp8A	-----S2-----S3-----		
htp8B	MVLVTVQARLISASGEVPMSEAVVLEHCNVVFARGPOMLEPFTITQRMFGDMREC		494
htp8B	MVLVTVQARLISASGEVPMSEAVVLEHCNVVFARGPOMLEPFTITQRMFGDMREC		494
Vr1	FMVSVLYFSQKRYASHMVEFLAMQNDMLTYTREGQOMGIYAMLMERHLDLQREN		582
ECaC	LWLTNQLRLNNGEYVPLSEAVVLEHCNVVFARGPOMLEPFTITQRMFGDMREC		494
htp8A	-----S4-----		
htp8A	WMAVTVLSEASAFYIIFQTED---FEE-----LG-HFYDYPMALSTELV		538
htp8B	WMAVTVLSEASAFYIIFQTED---FEE-----LG-HFYDYPMALSTELV		538
Vr1	FVYLAVLEISTAVVTLIEDGKNLSDESTPHKCRGSACKHNSYNSLYSTCLELHFT		642
ECaC	WMAVTVLSEASAFYIIFQTED---FNN-----LG-EFSDYPTALSTELF		538
htp8A	-----S5-----		
htp8A	LTITDGPANVWVLPENYSITVAFAIATLQALILYMHGQHWVRVHERDELARAI		598
htp8B	LTITDGPANVWVLPENYSITVAFAIATLQALILYMHGQHWVRVHERDELARAI		598
Vr1	IGMGLDETENDYFQAVFILLNVAFLTYILANLSEALGQVKNKIGESKNIRKLR		702
ECaC	LTITDGPANVWVLPENYSITVAFAIATLQALILYMHGQHWVRVHERDELARAI		598
htp8A	-----S6-----		
htp8A	VATVMTLRLPRCLNP---RSC---ICREYGLGQ---KRTFLVEDRODIARQRIQRYAQ		671
htp8B	VATVMTLRLPRCLNP---RSC---ICREYGLGQ---KRTFLVEDRODIARQRIQRYAQ		671
Vr1	ALITLDEKFLKMKRKAFFSKLQVGFDPGQDYKRCRVEDVNTWTTNNGIINE		762
ECaC	VATVMTLRLPRCLNP---RSC---ICREYGLGQ---KRTFLVEDRODIARQRIQRYAQ		671
htp8A	FHTR---SEDLKDSV-EKLELCPPSPHLSLPSVSRSTSRSSANWERLROGTLAR		726
htp8B	FHTR---SEDLKDSV-EKLELCPPSPHLSLPSVSRSTSRSSANWERLROGTLAR		726
Vr1	DPGN---CEGVKRTLSFSLRGRVSRNKNFALVPLRDASTRDRHATQEEVQLKHYTG		820
ECaC	FKCSKEDGQQLSEKRP-STVESQMLSRASVAFQTPSLRSTTSQSN-SHRGWELLAR		728
htp8A	DLRGIINRGLDGESWEYQI*		746
htp8B	DLRGIINRGLDGESWEYQI*		746
Vr1	SLKPEDAEVTKSMVPEK*		839
ECaC	NTLGLNLGLDLGEGDGEVYH*		751

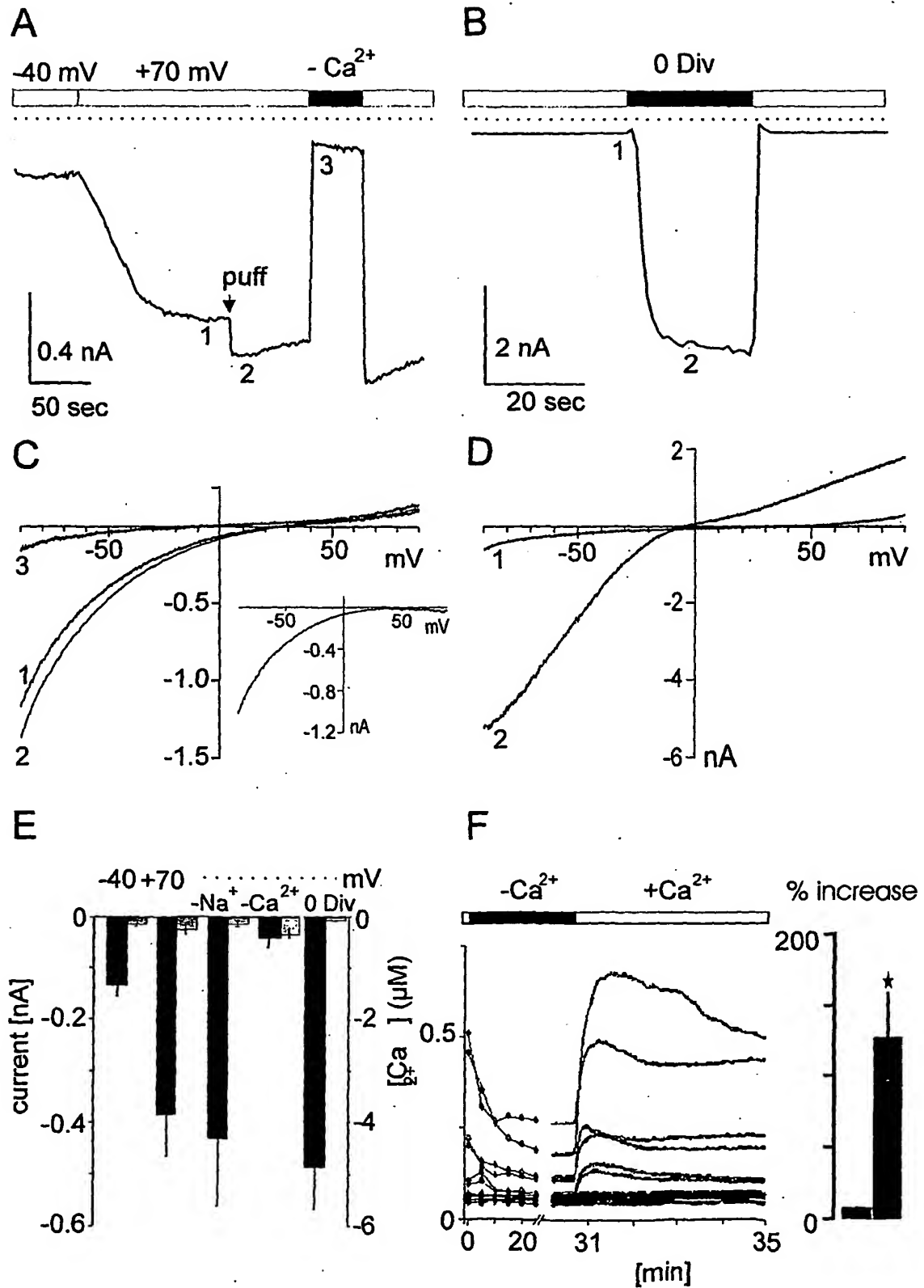
Figs. 2A and 2B



Figs. 2C and 2D

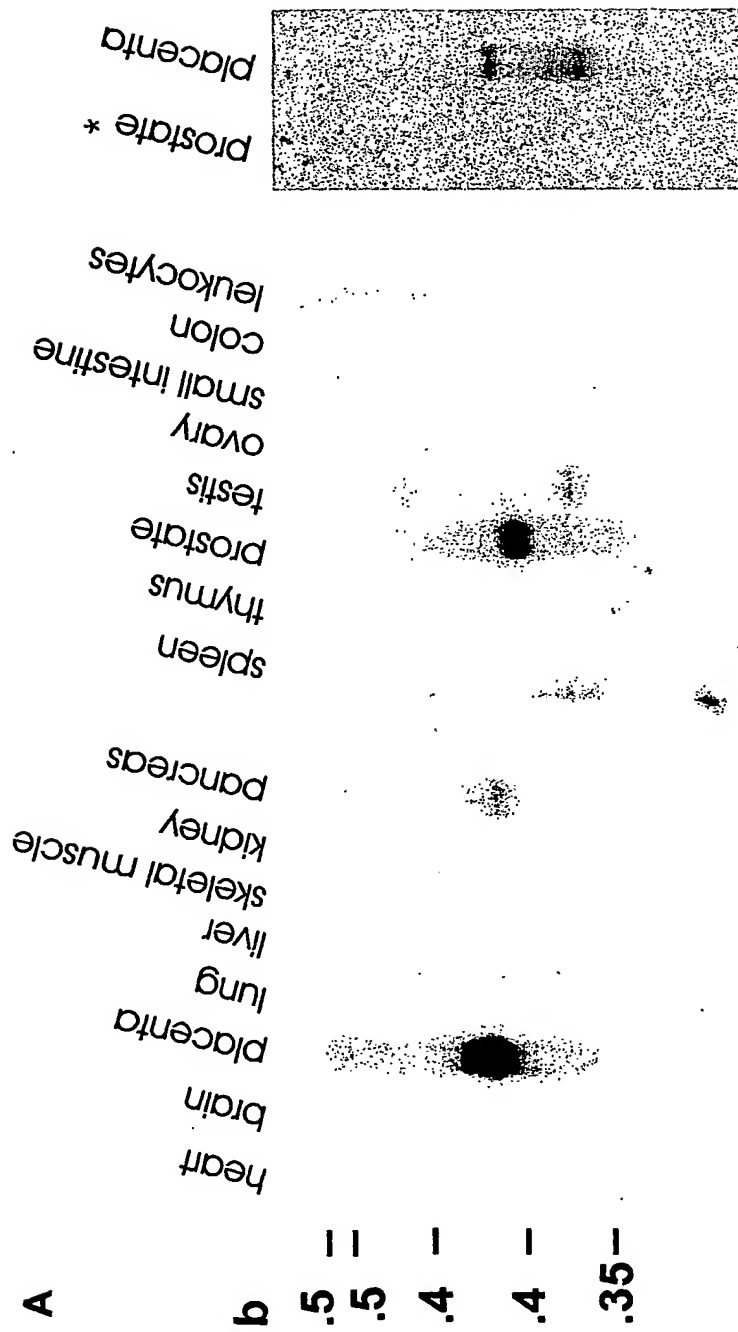


Figs. 3A – 3F

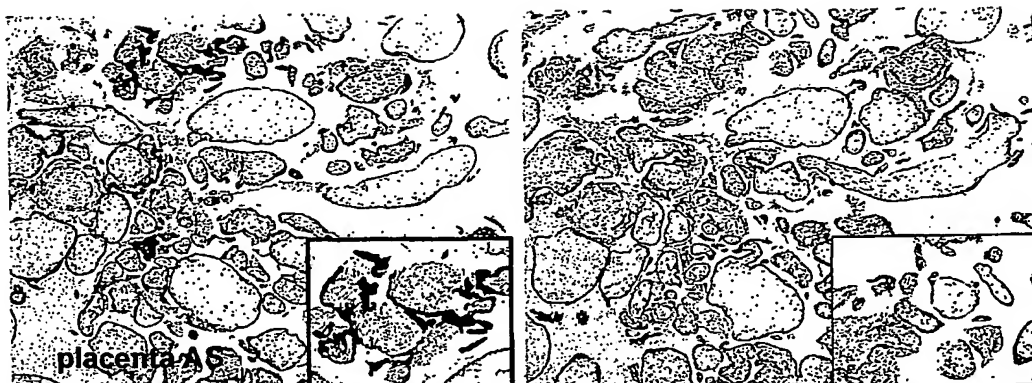
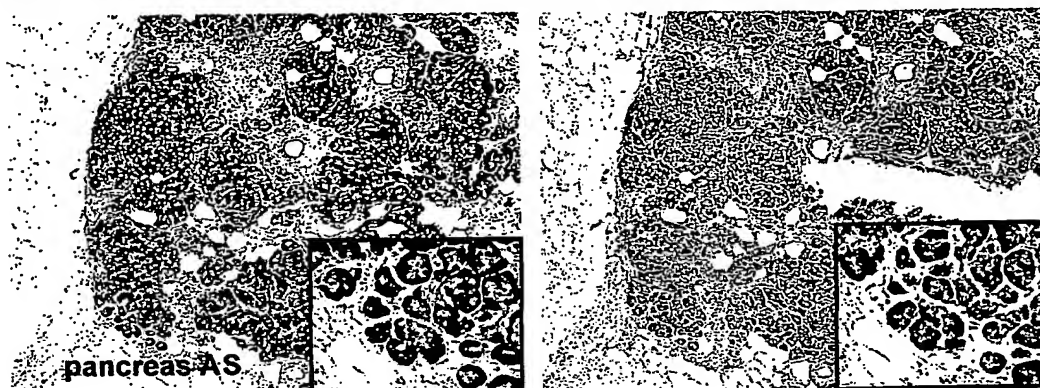
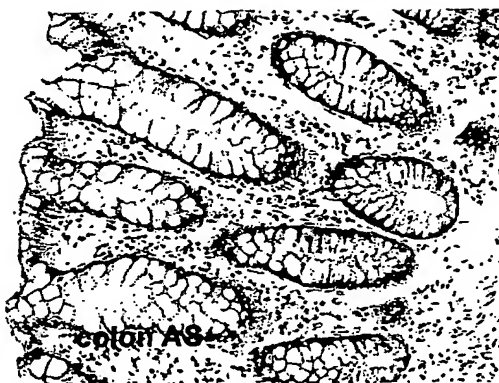


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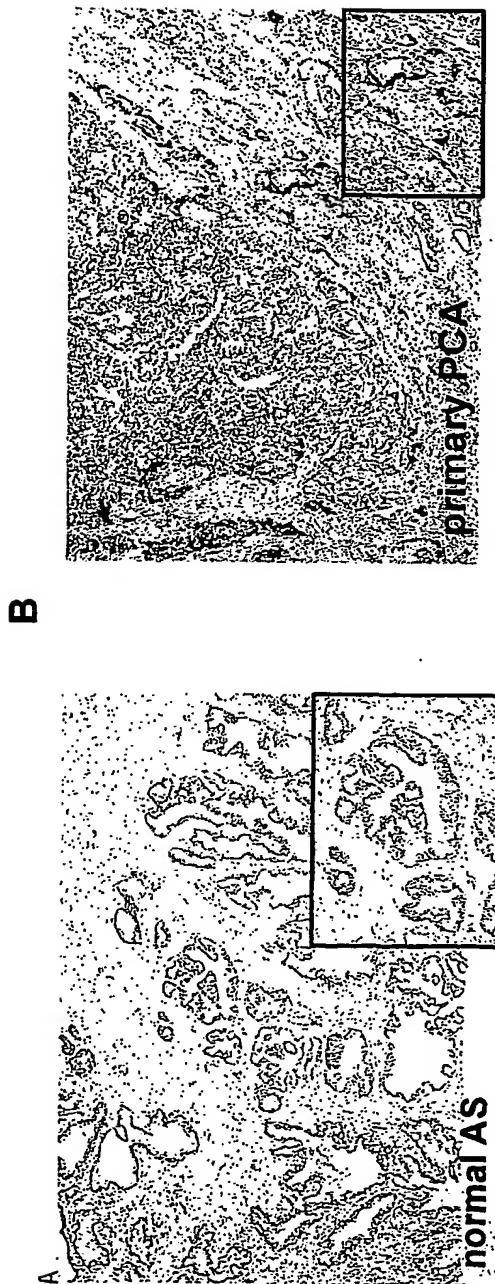
Fig. 5A



Figs. 5B – 5D

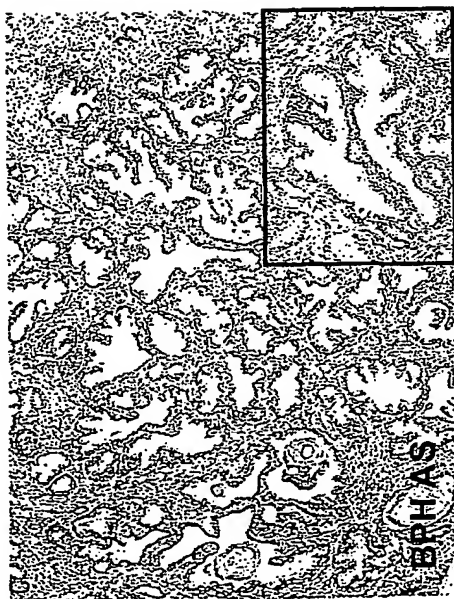
B**C****D**

Figs. 6A and 6B



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Figs. 6C - 6F



D



E



F



Fig. 7

```

10          30          50
GCCAAGTGTAACTCACTACAGCCCTCTCCAACTGGCTGGGGCTGCTGGGAGACTCCCA
70          90          110
AGGAAGTCTGTAGGAAGGCAGGAGACAGGAGACGGGACCTCTACAGGGAGACGGTGGGCC
130         150         170
GGCCCTTGGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGCTCTGGCCTCGGCC
190         210         230
TCAGGCCCCCAAGGAGCCGGCCCTACACCCCATGGGTTTGTCACTGCCCAAGGAGAAAGG
          M G L S L P K E K G
250         270         290
GCTAATTCTCTGCCTATGGAGCAAGTTCTGCAGATGGTTCCAGAGACGGGAGTCTGGGG
L I L C L W S K F C R W F Q R R E S W A
310         330         350
CCAGAGCCGAGATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGGAGTCTCCTCTCCT
Q S R D E Q N L L Q Q K R I W E S P L L
370         390         410
TCTAGCTGCCAAGATAATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTG
L A A K D N D V Q A L N K L L K Y E D C
430         450         470
CAAGGTGCACAGAGAGGAGCCATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGA
K V H Q R G A M G E T A L H I A A L Y D
490         510         530
CAACCTGGAGGCGCCATGGTGTGATGGAGGCTGCCCCGGAGCTGGTCTTTGAGCCCAT
N L E A A M V L M E A A P E L V F E P M
550         570         590
GACATCTGAGCTCTATGAGGGTCAGACTGCACTGCACATCGCTGTTGTGAACAGAAACAT
T S E L Y E G Q T A L H I A V V N Q N M
610         630         650
GAACCTGGTGCAGAGCCCTGCTTGCCCGCAGGGCCAGTGTCTCTGCCAGAGCCACAGGCAC
N L V R A L L A R R A S V S A R A T G T
670         690         710
TGCCTTCCGCCGTAGTCCCGCAACCTCATCTACTTTGGGGAGCACCCCTTTGCTCCTTTGC
A F R R S P R N L I Y F G E H P L S F A
730         750         770
TGCCTGTGTGAACAGTGAGGAGATCGTGCGGCTGCTCATTGAGCATGGAGCTGACATCCG
A C V N S E E I V R L L I E H G A D I R
790         810         830
GGCCCAGGACTCCCTGGGAAACACAGTGTACACATCCTCATCCTCCAGCCCAACAAAC
A Q D S L G N T V L H I L I L Q P N K T
850         870         890
CTTTGCCTGCCAGATGTACAACCTGTTGCTGTCTACGACAGACATGGGGACCACTGCA
F A C Q M Y N L L L S Y D R H G D H L Q
910         930         950
GCCCTGGGACCTCGTGCCCAATCACCAGGGTCTCACCCTTTCAAGCTGGCTGGAGTGGGA
P L D L V P N H Q G L T P F K L A G V E
970         990         1010
GGGTAACTGTGATGTTTCAGCACCTGATGCAGAAGCGGAAGCACACCCAGTGGACGTA
G N T V M F Q H L M Q K R K H T Q W T Y
1030        1050        1070
TGGACCACTGAOCTCGACTCTCTATGACCTCACAGAGATCGACTCCTCAGGGGATGAGCA
G P L T S T L Y D L T E I D S S G D E Q
1090        1110        1130
GTCCCTGGGAACTTATCATCACCACCAAGAAGCGGGAGGCTCGCCAGATCCTGGACCA
S L L E L I I T T K K R E A R Q I L D Q
1150        1170        1190
GACGCGGTGAAGGAGCTGGTGAGCCTCAAGTGAAGCGGTACGGGCGGCCGTACTTCTG
T P V K E L V S L K W K R Y G R P Y F C
1210        1230        1250
CATGCTGGGTGCCATATATCTGCTGTACATCATCTGCTTCACCATGTGCTGCATCTACCG
M L G A I Y L L Y I I C F T M C C I Y R
1270        1290        1310

```

CCCCCTCAAGCCAGGACCAATAACCGCACAGCCCCGGGACACACCCCTCTTACAGCA
P L K P R T N N R T S P R D N T L L Q Q
1330 1350 1370
GAAGCTACTTCAGGAAGCCTACGTGACCCCTAAGGACGATATCCGGCTGGTGGGGAGCT
K L L Q E A Y V T P K D D I R L V G E L
1390 1410 1430
GGTGACTGTCTTGGGCTATCATCATCTGCTGGTAGAGGTTCCAGACATCTTCAGAAT
V T V I G A I I I L L V E V P D I F R M
1450 1470 1490
GGGGGTCACTCGCTTCTTTGGACAGACCATCCTTGGGGGCCCATTCATGTCTCATCAT
G V T R F F G Q T I L G G P F H V L I I
1510 1530 1550
CACCTATGCCTTCATGGTGTGGTGACCATGGTGATGCGGCTCATCAGTGCCAGCGGGGA
T Y A F M V L V T M V M R L I S A S G E
1570 1590 1610
GGTGGTACCCATGTCTTTCGCACTCGTGGCTGGTGCACGTCATGTACTTCGCCCCG
V V P M S F A L V L G W C N V M Y F A R
1630 1650 1670
AGGATTCCAGATGCTAGGCCCTTCACCATCATGATTGAGAAGATGATTTTGGCGACCT
G F Q M L G P F T I M I Q K M I F G D L
1690 1710 1730
GATGOGATTCTGCTGGCTGATGGCTGTGGTCATCTGGGCTTTGCTTCAGCCTTCTATAT
M R F C W L M A V V I L G F A S A F Y I
1750 1770 1790
CATCTTCCAGACAGAGGACCCCGAGGAGCTAGGCCACTTCTACGACTACCCCATGGCCCT
I F Q T E D P E E L G H F Y D Y P M A L
1810 1830 1850
GTTTCAGCACCTTCGAGCTGTTCTTACCATCATCGATGGGCCAGCCAACTACAACGTGGA
F S T F E L F L T I I D G P A N Y N V D
1870 1890 1910
CCTGCCCTTCATGTACAGCATCACCTATGCTGCCTTTGCCATCATCGCCACACTGCTCAT
L P F M Y S I T Y A A F A I I A T L L M
1930 1950 1970
GCTCAACCTCCTCATTGCCATGATGGGCGACACTCACTGGCGAGTGGCCCATGAGCGGGA
L N L L I A M M G D T H W R V A H E R D
1990 2010 2030
TGAGCTGTGGAGGGCCAGATTGTGGCCACCAOGGTGATGCTGGAGCGGAAGCTGCCTCG
E L W R A Q I V A T T V M L E R K L P R
2050 2070 2090
CTGCCTGTGGCCTCGCTCOGGGATCTGCGGACGGGAGTATGGCCTGGGGGACCGCTGGTT
C L W P R S G I C G R E Y G L G D R W F
2110 2130 2150
CCTGCGGGTGGGAAGACAGGCAAGATCTCAACCGGCAGCGGATCCAACGCTACGCACAGGC
L R V E D R Q D L N R Q R I Q R Y A Q A
2170 2190 2210
CTTCCACACCCGGGGCTCTGAGGATTTGGACAAAGACTCAGTGGAAAACTAGAGCTGGG
F H T R G S E D L D K D S V E K L E L G
2230 2250 2270
CTGTCCCTTCAGCCCCACCTGTCCCTTCTACGCCCTCAGTGTCTCGAAGTACCTCCCCG
C P F S P H L S L P T P S V S R S T S R
2290 2310 2330
CAGCAGTGCCAATTGGGAAGGCTTCGGCAAGGGACCCTGAGGAGAGACCTGCGTGGGAT
S S A N W E R L R Q G T L R R D L R G I
2350 2370 2390
AATCAACAGGGGTCTGGAGGACGGGGAGAGCTGGGAATATCAGATCTGACTGCGTGTCT
I N R G L E D G E S W E Y Q I
2410 2430 2450
CACTTCGCTTCCCTGGAACCTTGCTCTCATTTTCTGGGTGCATCAACAAAAACAAAACCA
2470 2490 2510
AACCCAGAGGTCTCATCTCCAGGCCCGAGGAGAAAGAGGAGTAGCATGAACGCCAA
2530 2550 2570
GGAATGTACGTTGAGAATCACTGCTCCAGGCCCTGCATTACTCCTTCAGCTCTGGGGCAGA

Fig. 7 / continuation 2

```
2590          2610          2630
GGAAGCCCAGCCCAAGCACGGGGCTGGCAGGGCGTGAGGAACTCTCCTGTGGCCTGCTCA
2650          2670          2690
TCACCCCTTCCGACAGGAGCACTGCATGTCAGAGCACTTTAAAAACAGGCCAGCCTGCTTG
2710          2730          2750
GGCCCTCGGTCTCCACCCAGGGTCATAAGTGGGGAGAGAGCCCTTCCCAGGGCACCAG
2770          2790          2810
GCAGGTGCAGGGAAGTGCAGAGCTTGTGGAAGCGTGTGAGTGAGGGAGACAGGAACGGC
2830          2850          2870
TCTGGGGGTGGGAAGTGGGGCTAGGTCTTGCCAACTCCATCTTCAATAAAGTCGTTTTCG
2890          2910
GATCCCTAAAAA
```

MGLSLPKEKGLILCLWSKFCRWFQRRESWAQSRDEQNLLQOKRIWESPLLLAAKDNVDVQALNKLKLYEDCKVHQRGAMGETALHIA
ALYDNLEAMVLMEEAPELVFEPMTSELYEGQTALHIAVNVQNMNLVRALLARRASVSARATGTAFRRSPRNLIYFGEHPLSFAAC
VNSEIIVRLIEHGADIRAQDSLNTVLHILILQPNKTFACQMYNLLSYDRHGDHLQPLDLVPNHQGLTPFKLAGVEGNTVMFQH
LMQKRKHTQWTYGPLTSTLYDLTEIDSSGDEQSLELIITTKKREARQILDQTPVKELVSLKWKRYGRPYFCMLGAIYLLYIICFT
MCCIYRPLKPTNNRTSPRDNLTLLQOKLLEAYVTPKDDIRLVGELVTVIGAIILLVEVPDIFRMGVTRFFGQTLGGPFHVLII
TYAFMVLVTMVMRLISASGEVVPMSFALVLGWCNVMYFARGFQMLGPFTIMI QKMFQDLMRFCWLMAVVILGFASAFYIIFQTED
PEELGHFYDYPMALFSTFELFTIIDGPANYNDLPFMYSTIYAAFATLLMLNLLIAMMGDTHWRVAHERDELWRAQIVATTV
MLERKLPRCLWPRSGICGREYGLDRWFLRVEDRQDLNRQRIQRYAQAFHTRGSEDLDKDSVEKLELGCPFSPHLSLPTPSVSRST
SRSSANWERLRQGTLRRLRGIINRGLEDGESWEYQI

Figure 8:

A)

```
ATGGGTTTGTCACTGCCCAAGGAGAAAGGGCTAATTCTCT
M G L S L P K E K G L I L C
250      270      290
GCCTATGGAGCAAGTTCTGCAGATGGTTCCAGAGACGGGAGTCCTGGGCCCAGAGCCGAG
L W S K F C R W F Q R R E S W A Q S R D
310      330      350
ATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGGAGTCTCCTCTCCTTCTAGCTGCCA
E Q N L L Q Q K R I W E S P L L L A A K
370      390      410
AAGATAATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTGCAAGGTGCACC
D N D V Q A L N K L L K Y E D C K V H Q
430      450      470
AGAGAGGAGCCATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACAACCTGGAGG
R G A M G E T A L H I A A L Y D N L E A
490      510      530
CCGCCATGGTGTGATGGAGGCTGCCCCGGAGCTGGTCTTTGAGCCCATGACATCTGAGC
A M V L M E A A P E L V F E P M T S E L
550      570      590
TCTATGAGGGTCAGACTGCACTGCACATCGCTGTTGTGAACCAGAACATGAACCTGGTGC
Y E G Q T A L H I A V V N Q N M N L V R
610      630      650
GAGCCCTGCTTGCCCGCAGGGCCAGTGTCTTGCCAGAGCCACAGGCACTGCCTTCCGCC
A L L A R R A S V S A R A T G T A F R R
670      690      710
GTAGTCCCTGCAACCTCATCTACTTTGGGGAGCACCCCTTTGTCCTTTGCTGCCTGTGTGA
S P C N L I Y F G E H P L S F A A C V N
```

Fig. 8 / continue n 1

730 750 770
ACAGTGAGGAGATCGTGCAGCTGCTCATTGAGCATGGAGCTGACATCCGGGCCAGGACT
S E E I V R L L I E H G A D I R A Q D S
790 810 830
CCCTGGGAACACAGTGTACACATCCTCATCCTCCAGCCCAACAAACCTTTGCCTGCC
L G N T V L H I L I L Q P N K T F A C Q
850 870 890
AGATGTACAACCTGTTGCTGTCTACGACAGACATGGGGACCACCTGCAGCCCTGGACC
M Y N L L L S Y D R H G D H L Q P L D L
910 930 950
TCGTGCCCAATCACCAGGCTCTACCCTTTCAAGCTGGCTGGAGTGGAGGGTAACACTG
V P N H Q G L T P F K L A G V E G N T V
970 990 1010
TGATGTTTCAGCACCTGATGCAGAAGCGGAAGCACACCCAGTGCAGCTATGGACCACTGA
M F Q H L M Q K R K H T Q W T Y G P L T
1030 1050 1070
CCTCGACTCTCTATGACCTCACAGAGATCGACTCTCAGGGGATGAGCAGTCCCTGCTGG
S T L Y D L T E I D S S G D E Q S L L E
1090 1110 1130
AACTTATCATCACCACCAAGAAGCGGGAGGCTGCCAGATCCTGGACCAGACGCCGGTGA
L I I T T K K R E A R Q I L D Q T P V K
1150 1170 1190
AGGAGCTGGTGAGCCTCAAGTGGGAAGCGGTACGGGCGGCGTACTTCTGCATGCTGGGTG
E L V S L K W K R Y G R P Y F C M L G A
1210 1230 1250
CCATATATCTGCTGTACATCATCTGCTTCACCATGTGCTGCATCTACCGCCCCCTCAAGC
I Y L L Y I I C F T N C C I Y R P L K P
1270 1290 1310
CCAGGACCAATAACCGCACGAGCCCCCGGGACAACACCCCTCTTACAGCAGAAGCTACTTC
R T N N R T S P R D N T L L Q Q K L L Q
1330 1350 1370
AGGAAGCCTACATGACCCCTAAGGACGATATCCGGCTGGTGGGGAGCTGGTCACTGTCA
E A Y M T P K D D I R L V G E L V T V I
1390 1410 1430
TTGGGGCTATCATCATCTGCTGGTAGAGTTCCAGACATCTTCAGAATGGGGGTCACTC
G A I I I L L V E V P D I F R M G V T R
1450 1470 1490
GCTTCTTTGGACAGACCATCCTTGGGGGCCCATTCATGTCTCTATCATCACCTATGCCT
F F G Q T I L G G P F H V L I I T Y A F
1510 1530 1550
TCATGGTGTGGTGACCATGGTGTATGCGGCTCATCAGTGCCAGCGGGAGGTGGTACCCA
M V L V T M V M R L I S A S G E V V P M
1570 1590 1610
TGTCCTTTGCACTCGTGTGGGCTGGTGCAACGTATGTACTTCGCCCGAGGATTCCAGA
S F A L V L G W C N V M Y F A R G F Q M
1630 1650 1670
TGCTAGGCCCTTCACCATCATGATTCAGAAGATGATTTTGGCGACCTGATGCGATTCT
L G P F T I M I Q K M I F G D L M R F C
1690 1710 1730
GCTGGCTGATGGCTGTGGTCATCCTGGGCTTTGCTTCAGCCTTCTATATCATCTTCCAGA
W L M A V V I L G F A S A F Y I I F Q T
1750 1770 1790
CAGAGGACCCCGAGGAGCTAGGCCACTTCTACGACTACCCCATGGCCCTGTTTCAGCACCT
E D P E E L G H F Y D Y P M A L F S T F
1810 1830 1850
TCGAGCTGTTCTTACCATCATCGATGGCCCGCCAACTACAACGTGGACCTGCCCTTCA
E L F L T I I D G P A N Y N V D L P F M
1870 1890 1910
TGTAAGCATCACCTATGCTGCCTTTGCCATCATCGCCCACTGCTCATGCTCAACCTCC
Y S I T Y A A F A I A T L L M L N L L
1930 1950 1970
TCATGCCATGATGGGCGACACTCACTGGCGAGTGGCCCATGAGCGGGATGAGCTGTGGA

Fig. 8 / continué on 2

```

      I A M M G D T H W R V A H E R D E L W R
      1990                2010                2030
GGGCCCAGATTGTGGCCACCACGGTGATGCTGGAGCGGAAGCTGCCTCGCTGCCTGTGGC
      A Q I V A T T V M L E R K L P R C L W P
      2050                2070                2090
CTCGCTCCGGGATCTGCGGACGGGAGTATGGCCTGGGAGACCGCTGGTTOCTGCGGGTGG
      R S G I C G R E Y G L G D R W F L R V E
      2110                2130                2150
AAGACAGGCAAGATCTCAACGGCAGCGGATCCAACGCTACGCACAGGCCTTCCACACCC
      D R Q D L N R Q R I Q R Y A Q A F H T R
      2170                2190                2210
GGGGCTCTGAGGATTTGGACAAAGACTCAGTGGAAAACTAGAGCTGGGCTGTCCCTTCA
      G S E D L D K D S V E K L E L G C P F S
      2230                2250                2270
GCCCCCACCCTGTCCCTTCTATGCCTCAGTGTCTCGAAGTACCTCCCGCAGCAGTGCCA
      P H L S L P M P S V S R S T S R S S A N
      2290                2310                2330
ATTGGGAAAGGCTTCGGCAAGGGACCTGAGGAGAGACCTGCGTGGGATAATCAACAGGG
      W E R L R Q G T L R R D L R G I I N R G
      2350                2370                2390
GTCTGGAGGACGGGAGAGCTGGGAATATCAGATCTGA
      L E D G E S W E Y Q I *

```

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 ALYDNLEAAMVLMFAPELVFEPMTSELYEGQTALHIAVNVQNMNLVRALLARRASVSARATGTAFRRSPCNLIYFGEHPLSFAAC
 VNSEEIVRLLEHGA DIRAQDSLNTVLHILILQPNKTFACQMYNLLSYDRHGDHLOPLDLVPHQGLTPFKLAGVBGNTVMFQD
 LMQKRKHTQWYGPLTSTLYDLTEIDSSGDEQSLELIITTKREARQILDQTFVKELVSLKWKRYGRPYFCMLGAIYLLYIICFT
 MCCIYRPLKPRNTNRTSPRDNLLQOKLLOEAYMTPKDDIRLVGELVTIVIGAIILVEVPDIERMGVTRFFGQTLILGGPFHVLI
 TYAFMVLTVMVRLISASGEVVPMSFALVLGWCNVMYFARGFQMLGPFTIMIQKMIFGDLMRFCWLMMAVVLGFASAFYIIIFQTED
 PEELGHFYDYPMALFSTFEFLTIIDGPANYVDLPFMYSTIYAAFAIATLMLNLLIAMMGDTHWRVAHERDELWRAQIVATT
 MLERKLPRCLNFRSGTCGREYGLGDRWFLRVEDRQDLNRQIRIQRYAQAEHTRGSEDLKDKDSVERLELGCFFSPHLSLPMPSVSRST
 SRSSANWERLQGTLLRDLRGIINRGLEDGESWEYQI

B)

CAAACTCACAGCCCTCTCCAACTGGCTGGGGCTGCTGGGAGACTCCCAAGGAACTCGTCAGGAAGGCAGGAGACAGGAGACGGGA
 CCTCTACAGGGAGACGGTGGGCCCGGCCCTTGGGGGGGCTGATGTGGCCCAAGGCTGAGTCCCGTCAGGCTCTGGCCTCGGCCCTCA
 GGCCCCAAGGAGCCGCGCCCTACACCCCATGGGTTGTGCTACTGCCCAAGGAGAAAGGGCTAATCTCTGCCTATGGAGCAAGTTCT
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 GCCCTGCTTGCCCGCAGGGCCAGTGTCTGTCAGAGGCCACAGGCACTGCCTTCCGCCGTAGTCCCGCAACCTCATCTACTTTGG
 GGAGCACCTTTGTCTTTGTCTGCTGTGTGAACAGTGAGGAGATCGTGGCGGTGCTCATTGAGCATGGAGCTGACATCCGGGCCC
 AGGACTCCCTGGCCCAACAAACCTTTGCTGCCAGATGTACAACCTGTTGCTGTCTAOGACAGACATGGGGACCACTGCAAGCC
 CCTGGACCTCGTGCCCAATCACAGGGTCTCACCCCTTTCAAGCTGGCTGGAGTGGAGGGTAACACTGTGATGTTTCAGCACCTGA
 TGCAGAAGCGGAGACACACCCAGTGGAGCTATGGACCACTGACCTCGACTCTCTATGACCTCACAGAGATCGACTCTTCAGGGGAT
 GAGCAGTCCCTGCTGGAACCTTATCATCACCAAGAAGCGGGAGGCTCGCCAGATCTGGACAGAGCGCGGTGAAGGAGCTGGT
 GAGCCTCAAGTGAAGCGGTACGGCGGGCGGTACTTCTGCATGCTGGGTGCCATATATCTGCTGTACATCATCTGCTTCACCATGT
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 GAAGCCTACGTGACCCCTAAGGACGATATCCGGCTGGTGGGGAGCTGGTGACTGTCTATTGGGGCTATCATCATCTGCTGGTAGA
 GGTTCAGACATCTTCAGAAATGGGGTCACTCGCTTCTTTGGACAGACCATCTTGGGGGCCATTCATGCTCTCATCATCACT
 ATGCTTTCATGGTGTGGTGACCATGGTGATGCGGCTCATATGATTTTGGCGACCTGATGCGATCTGCTGGCTGATGGCTGG
 TCCTCTGGGCTTTGCTTCAAGCTTCTATATCATCTTCCAGACAGAGGAGGCCAGGAGCTAGGCCACTTCTACGACTACCCCATG
 CCCTGTTTCAGACACCTTCGAGCTGGTCTTACCATCATCGATGGCCAGCAACTACAACGTGGACCTGCCCTTCATGTACAGCAT
 CACCTATGCTGCCTTTGCCATCATCGCCCACTGCTCATGCTCAAOCTCTCATTTGOCATGATGGGCGCACTCACTGGGAGTGG
 CCGATGAGCGGGATGAGCTGTGGAGGGCCAGATTGTGGCCACCAAGGTGATGCTGGAGCGGAAGCTGCCCTGCTGCTGTGGCT
 CGCTCCGGGATCTGCGGACGGGAGTATGGCCTGGGGACCGCTGGTTCCTGCGGGTGAAGACAGGCAAGATCTCAACCGGACGCG

c.)

D.)

CAAACTCAGAGCCCTCTCAAACCTGGCTGGGGCTGCTGGGAGACTCCCAAGGAACCTCGTCAGGAAGGCAGGAGACAGGAGACGGGA
CCTCTACAGGGAGACGGTGGGCCGGCCCTTGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGCTCTGGCCTCGGCCCTCA
GGCCCCCAAGGAGCCGGCCCTACACCCCATGGTTTGCTACTGCCAAGGAGAAAGGGCTAAATCTCTGCCCTATGAGACAAGTTCT
GCAGATGTTCCAGAGACGGGAGTCTCTGGGCCAGAGCCGAGATGAGCAGAACTGCTGCAGCAGAAAGAGGATCTGGGAGTCTCCT
CTCCTTCTAGCTGCCAAAGATATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTGCAAGGTGCACCAGAGAGGAGC
CATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACAACTGGAGGCCCGCATGGTGCTGATGGAGGCTGCCCCGAGCTGG
TCTTTGAGCCCATGACATCTGAGCTCTATGAGGGTCAAGTGCATGCATCGCTGTTGTGAACAGAAAGTCAACTGAACCTGGTGCGA
GCCCTGTGCTCCCGCAGGGCCAGTGTCTCTGCGAGAGCCACAGGCATGCTGCTTCCGCGTAGTCCCGCAACCTCATCTACTTTGG

E.)

CACACATGEGGCCCTCCCAGGAGTGCCCAGGAACCTCGTGCTGTTGGCCCTCTGAATCTATOGTCTCCAATCOGCTGTCCCACAGAAGC
CATAAACCACCTCTCTGTAATGCCAGGAGCCATGGGGGAAACAGCGGTACACATAGCAGCCCTCTATGACAACCTGGAGGCCG
CCATGGTGCTGATGGAGGCTGCCCCGGAGCTGGTCTTTTGAAGCCCATGACATCTGAGCTCTATGGAGGGTGAGGGGCCACGGGTCTG
GGGTGAAGAGCAGGAGTGACGTGGTGGGATTTCAAGTCAGTCTCTGTGATGATGAATTTGGGAAAGCAGAGGGGATCTGAGCT
CCTACTCTTTTSTCTCTCTGTCTCCCTCCGTGTCAGTCCCTGACTGCCATFACATCTGAACGCTCTGCCCTCTGGAAATGCCAGG
GCTTAGAGAAAGAGAGAGATGGGCAGCAGCTGGATCCCTGGGAATCCTGAACAOCAGAGCTCCCTGTTTCTCCATCCAGGC
ACCCCTGAGGGAAAGAGACTAGGGGTGCATATGGGAGGGACCCCTGCGAGGATCTAGGGGACAGACCCGTGACTGACAGCTGTCT
CTGGGCCAGGTGACACTGCACTGCAATCGCTGTTGTGAACCAGAACATGAACCTGCTGCGAGCCCTGCTTGCCCGCAGGGCCAGT
GTCTCTGCCAGAGCCACAGGCCTGCTTCCGCCGTAGTCCCTCGAACCTCATCTACTTTGGGGAGCACCCCTTTGTCTTTGCTGT
CTGTGTGAACACTGAGGAGATCGTGGCGCTCTCATTTGACATGGAGCTGACATCCGGGCCAGGACTCCCTGATGTACAACCTG
TTGCTGTCTCTACAGACAGATGGGGACAGCTGCGACCCCTGGACCTCGTGCCCAATCACCAGGGTCTACCCCTTTCAAGCTGGC
TGERGTGGAGGTAACACTGTGATGTTTACGACCTGATGCAGAAGCGGAAGCACACCCAGTGGACGATGGACCACTGACCTCGA
CTCTCTATGACCTCACAGAGATCGACTCTCAGGGGATGAGCAGTCCCTGCTGGAACCTTATCATCACCCACAGAGCGGGAGGCT
CGCCAGATCTTGACCCAGACGCCGTGAAGGAGCTGGTGAGCTCAAGTGGAAAGCGGTACGGGCGGCCGTACTTCTGCATGCTGGG
TGCCATATATCTGCTGTACATCATCTGCTTCACCATGTGCTGCATCTACCGCCCCCTCAAGCCACAGAACATAACCCGACGAGCC
CCGGGACCAACACCTCTTACAGCAGAAGCTACTTCAGGAGCTACATGACATCCCTAAGSAGGATATCCGCTGGTCCGGGAGCTG
GTGACTGTCACTTGGGGCTATCATCATCTCTGGTGAGAGTTCCAGACATCTCAGAAATGGGGTCACTCGCTTCTTTGGACAGAC
CATCTTGGGGGCCATTTCATGTCTCATCATCACCTATGCCTTCATGTGCTGGTGACCATGGTGATCGGGCTCATCAGTGCCA
GCGGGGAGGTGGTACCATGTCTTTGCACTCGTGCTGGGCTGGTGCAACGTCATGTACTTGCCTGAGGATTCAGATGTCTAGGC
CCCTTCACCATCATGATTCAGAGATGATTTTTGGCGACCTGATGCAATCTGCTGGCTGATGGCTGTGGTCACTCTGGGCTTTC
TTCAGCCTTCTATATCATCTTCCAGACAGAGGACCCCGAGGAGCTAGGCCACTTCTAGACTACCCCATGGCCCTGTTTCAGCACCT
TCGAGCTGGTCTTACCATCATCGACTGSCCCAGCCAACTACAACTGGACGCTGCCCTCATGTACAGCATCACCATGCTGCCCTT
GCCATCATCGCCACACTGCTCATGCTCAACCTCTCATTCGCTGATGGGCGACACTCATGGCGAGTGGCCCTATGAGCGGATGA
GCTGTGGAGGGCCAGATTGTGCGCCACCGGATGCTGGAGCGGAAGCTGCTGCTGCTGTGGCTCGCTCCGGGATCTGCG
GACGGGAGTATGGCTGGGAGACCGCTGGTTCCTGCGGGTGGAAGACAGGCAAGATCTCAACCGCGAGCGGATCCAACGCTACGCA
CAGGCTTCCACACCCGGGGCTCTGAGGATTGGACAAGAGCTCAGTGAAAAACTAGAGCTGGGCTGTCCCTTCAGCCCCACCT
GTCCCTTCTATGCCCTCAGTGTCTCGAAGTACCTCCCGCAGCAGTGCCAATTTGGAAAGGCTTCGCGAAGGGACCTTGAGGAGAG
ACCTGCGTGGGATAATCAACAGGGGTCTGGAGACGGGGAGAGCTGGGAATATCAGATCTGACTGCGTGTCTCACTTGCTTCT
GGAACCTGCTCTCATTTTCTGGGTGCATCAACAAAACAAAACCAACACCCAGAGGTCTCATCTCCAGGCCCCAGGGAGAA
GAGGAGTAGCATGAACGGGCTGACGTTGAGAACTCCTGCTCCAGGCTGCATTACTCTTCAGTCTTGGGCGAGGAGAG
CCAGCCCAAGCACGGGCCGAGGCGGTGAGGAATCTCTGCTGGCCGTGCTCATACCCCTCCGACAGGAGCATGCTCATGTGAG
AGCACTTTAAAAACAGGCGAGCCTGCTTGGGCCCTCGGTCTCCACCCAGGGTCATAGTGGGGAGAGAGCCCTTCCAGGGCACC

Fig. 8 / continuation 5

CAGGCAGGTGCAGGGAAGTGCAGAGCTTGTGGAAAGCGTGTGAGTGAGGGAGACAGGAACGGCTCTGGGGGTGGGAAGTGGGGCTA
GGTCTTGCCAACTCCATCTTCAATAAAGTCGTTTTTCGGATCCCTAAAAAAAAAAAAAAAAAAAAAAAAA

Figure 9:

A.

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      10              30              50
CGGGGCCCTGGGCTGCAGGAGGTTGCGGCGGCGCGGCAGCATGGTGGTGCCGGAGAAGG
                                M V V P E K E
      70              90              110
AGCAGAGCTGGATCCCAAGATCTTCAAGAAGAAGACCTGCACGACGTTCATAGTTGACT
  Q S W I P K I F K K K T C T T F I V D S
     130            150            170
CCACAGATCCGGGAGGGACCTTGTGCCAGTGTGGGCGCCCCCGGACCGCCAC'CCCGCAG
  T D P G G T L C Q C G R P R T A H P A V
     190            210            230
TGGCCATGGAGGATGCCTTCGGGGCAGCCGTGGTGACCGTGTGGGACAGCGATGCACACA
  A M E D A F G A A V V T V W D S D A R T
     250            270            290
CCACGGAGAAGCCCAACCGATGCCTACGGAGAGCTGGACTTCACGGGGGCGGGCCGAAGC
  T E K P T D A Y G E L D F T G A G R K H
     310            330            350
ACAGCAATTTCTCCGGCTCTCTGACCGAACGGATCCAGCTGCAGTTTATAGTCTGGTCA
  S N F L R L S D R T D P A A V Y S L V T
     370            390            410
CACGCACATGGGGCTTCCGTGCCCCGAACCTGGTGGTGTCA GTGCTGGGGGGATCGGGGG
  R T W G F R A P N L V V S V L G G S G G
     430            450            470
GCCCCGTCTCCAGACCTGGCTGCAGGACCTGCTGCGTCGTGGGCTGGTGCGGGCTGCC
  P V L Q T W L Q D L L R R G L V R A A Q
     490            510            530
AGAGCACAGGAGCCTGGATTGTCACTGGGGGTCTGCACACGGGCATCGGCCGGCATGTTG
  S T G A W I V T G G L H T G I G R H V G
     550            570            590
GTGTGGCTGTACGGGACCATCAGATGGCCAGCACTGGGGGCACCAAGGTGGTGGCCATGG
  V A V R D H Q M A S T G G T K V V A M G
     610            630            650
GTGTGGCCCCCTGGGGTGTGGTCCGGAATAGAGACACCCTCATCAACCCCAAGGGCTCGT
  V A P W G V V R N R D T L I N P K G S F
     670            690            710
TCCCTGCGAGGTACCGGTGGCGCGGTGACCCGGAGGACGGGGTCCAGTTTCCCCTGGACT
  P A R Y R W R G D P E D G V Q F P L D Y
     730            750            770
ACAACTACTCGGCCTTCTTCTGGTGGACGACGGCACACACGGCTGCCTGGGGGGCGAGA
  N Y S A F F L V D D G T H G C L G G E N
     790            810            830
ACCGCTTCCGCTTGCGCCTGGAGTCCTACATCTCACAGCAGAAGACGGGCGTGGGAGGGA
  R F R L R L E S Y I S Q Q K T G V G G T
     850            870            890
CTGGAATTGACATCCCTGTCTGCTCCTCCTGATTGATGGTGATGAGAAGATGTTGACGC
  G I D I P V L L L I D G D E K M L T R
     910            930            950
GAATAGAGAACGCCACCCAGGCTCAGCTCCCATGTCTCCTCGTGGCTCAGGGGGAG
  I E N A T Q A Q L P C L L V A G S G G A
     970            990            1010
CTGCGGACTGCCTGGCGGAGACCCTGGAAGACACTCTGGCCCCAGGGAGTGGGGGAGCCA
  A D C L A E T L E D T L A P G S G G A R
     1030            1050            1070
GGCAAGGCGAAGCCCAGATCGAATCAGGCGTTTCTTTCCCAAAGGGGACCTTGAGGTCC
```

Fig. 9 / continu: n 1

Q G E A R D R I R R F F P K G D L E V L
1090 1110 1130
TGCAGGCCCGAGGTGGAGAGGATTATGACCCGGAAGGAGCTCCTGACAGTCTATTCTTCTG
Q A Q V E R I M T R K E L L T V Y S S E
1150 1170 1190
AGGATGGGTCTGAGGAATTCGAGACCATAGTTTGAAGGCCCTTGTGAAGGCCTGTGGGA
D G S E E F E T I V L K A L V K A C G S
1210 1230 1250
GCTGGAGGCCCTCAGCCTACCTGGATGAGCTGCGTTTGGCTGTGGCTTGGAAACCGGTGG
S E A S A Y L D E L R L A V A W N R V D
1270 1290 1310
ACATTGCCAGAGTGAACCTCTTTCGGGGGACATCCAATGGCGGTCTTCCATCTCGAAG
I A Q S E L F R G D I Q W R S F H L E A
1330 1350 1370
CTTCCCTCATGGACGCCCTGCTGARTGACCGGCCTGAGTTCGTGGCTTGTCTCATTTCCC
S L M D A L L N D R P E F V R L L I S H
1390 1410 1430
ACGGCCTCAGCCTGGGCCACTTCTGACCCCGATGCGCCTGGCCCAACTCTACAGCGCGG
G L S L G H F L T P M R L A Q L Y S A A
1450 1470 1490
CGCCCTCCAACTCGCTCATCCGCAACCTTTTGGACCAGGCGTCCCACAGCGCAGGCACCA
P S N S L I R N L L D Q A S H S A G T K
1510 1530 1550
AAGCCCCAGCCCTAAAAGGGGGAGCTGCGGAGCTCGGGCCCCCTGACGTGGGGCATGTGC
A P A L K G G A A E L R P P D V G H V L
1570 1590 1610
TGAGGATGCTGCTGGGGAAGATGTGCGCGCGAGGTACCCCTCGGGGGCGCCTGGGACC
R M L L G K M C A P R Y P S G G A W D P
1630 1650 1670
CTCACCCAGGCCAGGGCTTCGGGAGAGCATGTATCTGCTCTCGGACAAGGCCACCTCGC
H P G Q G F G E S M Y L L S D K A T S P
1690 1710 1730
CGCTCTCGCTGGATGCTGGCCTCGGGCAGGCCCCCTGGAGCGACCTGCTTCTTTGGGCAC
L S L D A G L G Q A P W S D L L L W A L
1750 1770 1790
TGTGTGTAACAGGGCACAGATGGCCATGTACTTCTGGGAGATGGGTCCAATGCAGTTT
L L N R A Q M A M Y F W E M G S N A V S
1810 1830 1850
CCTCAGCTCTTGGGGCCTGTTTGTCTCGGGGTGATGGCACGCCCTGGAGCCTGACGCTG
S A L G A C L L L R V M A R L E P D A E
1870 1890 1910
AGGAGGCAGCACGGAGGAAGACCTGGCGTTCAAGTTTGAGGGGATGGGCGTTGACCTCT
E A A R R K D L A F K F E G M G V D L F
1930 1950 1970
TTGGCGAGTGCTATCGCAGCAGTGAGGTGAGGGCTGCCCGCCTCCTCCTCGCTCGCTGCC
G E C Y R S S E V R A A R L L L R R C P
1990 2010 2030
CGCTCTGGGGGGATGCCACTTGCCTCCAGCTGGCCATGCAAGCTGACGCCCCGTGCCCTTCT
L W G D A T C L Q L A M Q A D A R A F F
2050 2070 2090
TTGCCCAGGATGGGGTACAGTCTCTGCTGACACAGAAGTGGTGGGGAGATATGGCCAGCA
A Q D G V Q S L L T Q K W W G D M A S T
2110 2130 2150
CTACACCCATCTGGGCCCTGGTTCTCGCCTTCTTTTGGCCCTCCACTCATCTACACCCGCC
T P I W A L V L A F F C P P L I Y T R L
2170 2190 2210
TCATCACCTTCAGGAAATCAGAAGAGGAGCCACACGGGAGGAGCTAGAGTTTGACATGG
I T F R K S E E E P T R E E L E F D M D
2230 2250 2270
ATAGTGTCTAATAATGGGGAAGGGCTGTGCGGACGGCGGACCCAGCCGAGAAGACGCGC
S V I N G E G P V G T A D P A E K T P L
2290 2310 2330

Fig. 9 / continuation 2

TGGGGGTCCCGCCAGTCCGGCCGTCGGGGTTGCTGCGGGGGCCGCTGCGGGGGGCCGC
 G V P R Q S G R P G C C G G R C G G R R
 2350 2370 2390
 GGTGCCTACGCCGCTGGTTCCACTTCTGGGGCGTGCCGGTGACCATCTTCATGGSCAACG
 C L R R W F H F W G V P V T I F M G N V
 2410 2430 2450
 TGGTCAGCTACCTGCTGTTCTGCTGCTTTTCTCGCGGGTGCTGCTCGTGGATTTCACG
 V S Y L L F L L L F S R V L L V D F Q P
 2470 2490 2510
 CGCGCGCCGCCCGGCTCCCTGGAGCTGCTGCTCTATTTCTGGGCTTTCAOGCTGCTGTGCG
 A P P G S L E L L L Y F W A F T L L C E
 2530 2550 2570
 AGGAACATGCGCCAGGGCCTGAGOGGAGGCGGGGCGAGCCTCGCCAGCGGGGGCCCCGGGC
 E L R Q G L S G G G G S L A S G G P G P
 2590 2610 2630
 CTGGCCATGCCTCACTGAGCCAGCGCCTCGCGCTCTACCTCGCCGACAGCTGGAACCACT
 G H A S L S Q R L R L Y L A D S W N Q C
 2650 2670 2690
 GCGACCTAGTGGCTCTCACCTGCTTCTCTGCGCGTGCGGCTGACCCCCGGGTT
 D L V A L T C F L L G V G C R L T P G L
 2710 2730 2750
 TGTACCACCTGGGCGCACTGTCTCTGCTGACTTCATGGTTTTACGGTGCGGCTGCG
 Y H L G R T V L C I D F M V F T V R L L
 2770 2790 2810
 TTACACATCTTACGGTCAACAACAGCTGGGGCCAGATCGTCATCGTGAGCAAGATGA
 H I F T V N K Q L G P K I V I V S K M M
 2830 2850 2870
 TGAAGGACGTGTTCTTCTCTCTCTCTCTCTCGCGGTGTGGCTGGTATGCGCTATGGCGTGG
 K D V F F F L F F L G V W L V A Y G V A
 2890 2910 2930
 CCACGGAGGGGCTCCTGAGGOCACGGGACAGTGACTTOCCAAGTATCTGCGCGCGCTCT
 T E G L L R P R D S D F P S I L R R V F
 2950 2970 2990
 TCTACCGTCCCTACCTGCAGATCTTOGGGCGAGATTCCCGAGGAGACATGGACGTGGCCC
 Y R P Y L Q I F G Q I P Q E D M D V A L
 3010 3030 3050
 TCATGGAGCACAGCAACTGCTCGTGGGAGCCCGGCTTCTGGGACACCCCTCCTGGGGCCC
 M E H S N C S S E P G F W A H P P G A Q
 3070 3090 3110
 AGGCGGGCACCTGCGTCTCCAGTATGCCAACTGGCTGGTGGTGCTGCTCCTCGTCACT
 A G T C V S Q Y A N W L V V L L L V I F
 3130 3150 3170
 TCCTGCTCGTGGCCAACATCTGCTGGTCAACTTGCTCATTGCCATGTTCACTTACACAT
 L L V A N I L L V N L L I A M F S Y T F
 3190 3210 3230
 TCGGCAAGTACAGGGCAACAGCGATCTCTACTGGAAGGCGCAGCGTTACCGCCTCATCC
 G K V Q G N S D L Y W K A Q R Y R L I R
 3250 3270 3290
 GGGGAATTCACCTCTCGGCCCCGCGCTGCCCCCGCCCTTTATCGTCATCTCCCACTTGCGCC
 E F H S R P A L A P P F I V I S H L R L
 3310 3330 3350
 TCCTGCTCAGGCAATTTGTGCAGGCGACCCCGAGCCCCAGCCGTCCTTCCCCGGCCCTCG
 L L R Q L C R R P R S P Q P S S P A L E
 3370 3390 3410
 AGCATTTCCGGGTTTACCTTTCTTAAGGAAGCCGAGCGGAAGCTGCTACAGTGGAATCGG
 H F R V Y L S K E A E R K L L T W E S V
 3430 3450 3470
 TGCATAAGGAGAACTTTCTGCTGGCACGCGCTAGGGACAAGCGGGAGAGCGACTCCGAGC
 H K E N F L L A R A R D K R E S D S E R
 3490 3510 3530
 GTCTGAAGCGCACGTCCAGAAAGTGGACTTGGCACTGAAACAGCTGGGACACATCCGCG
 L K R T S O K V D L A L K O L G H I R E

Fig. 9 / continua 13

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3550      3570      3590
AGTACGAACAGCGCCTGAAAGTCTGGAGCGGGAGGTCCAGCAGTGTAGCCGCGTCTCTGG
  Y E Q R L K V L E R E V Q Q C S R V L G
3610      3630      3650
GGTGGGTGGCCGAGGCCCTGAGCGGCTCTGCCTTGCTGCCCCAGGTGGGCCGCCACCCC
  W V A E A L S R S A L L P P G G P P P P
3670      3690      3710
CTGACCTGCCTGGGTCCAAGACTGAGCCCTGCTGGCGGACTTCAAGGAGAAGCCCCCAC
  D L P G S K D *
3730      3750      3770
AGGGGATTTTGGCTCCTAGAGTAAGGCTCATCTGGGCGCTCGGCCCGCACCTGGTGGCCT
3790      3810      3830
TGTCTTGTAGGTGAGCCCCATGTCCATCTGGGCCACTGTGAGGACCACCTTTGGGAGTGT
3850      3870      3890
CATCCTTACAAACCACAGCATGCCCGGCTCCTCCAGAACCACTCCAGCCTGGGAGGAT
3910      3930      3950
CAAGGCCTGGATCCCGGGCCGTTATCCATCTGGAGGCTGCAGGCTCCTTGGGGTAACAGG
3970      3990      4010
GACCACAGACCCCTCACCCTCACAGATTCTCACACTGGGGAAATAAGCCATTTCAGA
4030
GGAAAAAAAAAAAAAAAAAAAA

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MVPPEKQSWIPKIEKKKTCTTFIVDSTDPGGTLQCQGRPRTAHPAVAMEDAFGAAVVTWVWSDAHTTEKPTDAYELDTGAGRKH
SNFLRLSDRTPAAVYSLVTRTWGFRAPNLVSVLGGSGGFVLQTLWQDLLRRGLVRAAQSTGAWIVTGGLHTGIGRHVGVAVRDH
QMASTGGTKVVMGVAPWGVVRNRDTLINPKGSFPARYRWRGDPEDGVQFPLDYNYSAFFLVDDGTHGCLGGENRFRRLRESYISQ
QKTGVGGTGIDIPVLLLLIDGDEKMLTRIENATQAHVPCILVAGSRGLGMPGGTLEAHLAQDGDHKAQSTNQLLLPKOLSLQFVE
SIDRKTLSQSYSERLAVAWNVRVDIAQSELFRGDIQWRSFHLEASLMDALLNDRPEFVRLLSHGLSLGHFLTFLMLAQLYSAAPSNS
LIRNLLDQASHSAGTKAPALKGGAAELRPPDVGHVLRMLLGKMCAPRYPSGGAWDPHPGQGFGESEMYLLSDKATSPLSLDAGLQOA
PWSDLLWALLNRAQMAFYFWEMGSNAVSSALGACILLRVMARLEPDAEEAARRKDLAFKTEGGMVDLFGECTYRSSEVRAARLLL
RRCPLMGDATCLQAMQADARAFAQDGVQSILTKWWDMASTPIWALVLAFFCPPLIYTRLITFRKSEEEPTREELEFDMDSV
INCEGPVGTADPAEKTPLGVPRQSGRPGCCGGRGCGGRRCLRRWFHFWGVPTIFMGNVVSYLELFLLLFSRVLLVDFQAPPGSLEL
LLYFWAFTLLCEELRQGLSGGGGSLASGGPGHASLSQRLRLYLADSWNQCDLVALTCFLLGVCRLTPLYHLGRTVLCIDFMV
FTVRLHLHIFTVNKQLGPKIVIVSKMKDVFVFFLFLGVWLVAVGATEGLLRPRDSDFPSILRRVFYRPLYQIFQIPQEDNDVAL
MEHSNCSSEPGFWAHPGAQAGTCVSQYANWLVLVLLVIFLLVANILLVNLLIAMFSYTFGKVQGNSDLYWKAQRYRLIREPHSRP
ALAPPFTIVISHRLRLRLQLCRPRSPQSPSPAHEFRVYLSKEAERKLLTWESVHKENFLARARDKRESDSLRLKRTSOKVDLAL
KQLGHIREYBQRLKVLREVVQCSRVLGWVAZALSRSALLPPGPPPPDLPGSKD

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B.)

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10      30      50
ATCCAATGGCGGTCTTCCATCTCGAAGCTTCCCTCATGGACGCCCTGCTGAATGACCGG
70      90      110
CCTGAGTTCGTGCGCTTGCTCATTTCCACGGCGCTCAGCCTGGGCCACTTCTGACCCCCG
130     150     170
ATGGCCTGGCCCCAACTCTACAGCGCGCGGCCCTCCAACCTGCTCATCCGCAACCTTTTG
190     210     230
GACCAGGCGTCCACAGCGCAGGCACCAAGCCCCAGCCCTAAAAGGGGGAGCTGCGGAG
250     270     290
CTCCGGCCCCCTGACGTGGGGCATGTGCTGAGGATGCTGCTGGGGAGATGTGCGCGCCG
310     330     350
AGATGTATCTGCTCTCGGACAAGGCCACCTCGCCGCTCTCGTGGATGCTGGCCTCGGGC
  M Y L L S D K A T S P L S L D A G L G Q
370     390     410
AGGCCCCCTGGAGCGACCTGCTTCTTTGGGCACTGTGCTGAACAGGGCACAGATGGCCA
  A P W S D L L L W A L L L N R A Q M A M
430     450     470
TGTACTTCTGGGAGATGGGTTCGAATGCAGTTTCTCAGCTCTTGGGGCCTGTTTGCTGC
  Y F W E M G S N A V S S A L G A C L L L

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Fig. 9 / continue 14

490 510 530
TCCGGGTGATGGCAGCGCTGGAGCCTGACGCTGAGGAGGAGCAGCAGGAGGAAAGACCTGG
R V M A R L E P D A E E A A R R K D L A
550 570 590
CGTTCAAGTTTGAGGGGATGGGCGTTGACCTCTTTGGCGAGTGCTATCGCAGCAGTGAGG
F K F E G M G V D L F G E C Y R S S E V
610 630 650
TGAGGGCTGCCCGCCTCCTCCTCCGTCGCTGCCCGCTCTGGGGGGATGCCACTTGCCCTCC
R A A R L L L R R C P L W G D A T C L Q
670 690 710
AGCTGGCCATGCAAGCTGACGCCCGTGCCTTCTTTGCCAGGATGGGGTACAGTCTCTGC
L A M Q A D A R A F F A Q D G V Q S L L
730 750 770
TGACACAGAAGTGTTGGGGAGATATGGCCAGCACTACACCCATCTGGGCCCTGGTTCTCG
T Q K W W G D M A S T T P I W A L V L A
790 810 830
CCTTCTTTTGGCCCTCCACTCATCTACACCCGCTCATCACCTTCAGGAAATCAGAAGAGG
F F C P P L I Y T R L I T F R K S E E E
850 870 890
AGCCACACGGGAGGAGCTAGAGTTTGACATGGATAGTGTCAATTAATGGGGAAGGGCCTG
P T R E E L E F D M D S V I N G E G P V
910 930 950
TCGGGACGGCGGACCCAGCCGAGAACGCCGCTGGGGGTCCCGCGCCAGTCCGGCCGCTC
G T A D P A E K T P L G V P R Q S G R P
970 990 1010
CGGGTTGCTCGGGGGCCGCTGCGGGGGCGCCGGTGCCTACGCCGCTGGTTCCACTTCT
G C C G G R C G G R R C L R R W F H F W
1030 1050 1070
GGGGCGTGCCGGTGACCATCTTCATGGGCAACGTGGTCAGCTACCTGCTGTTCTCTGCTGC
G V P V T I F M G N V V S Y L L F L L L
1090 1110 1130
TTTTCTCGCGGGTGCTGCTCGTGGATTTCAGCCGCGCCGCCCGCTCCCTGGAGCTGC
F S R V L L V D F Q P A P P G S L E L L
1150 1170 1190
TGCTCTATTCTGGGCTTTCACGCTGCTGTGCGAGGAAGTGGCCAGGGCCTGAGCGGAG
L Y F W A F T L L C E E L R Q G L S G G
1210 1230 1250
GGGGGGCAGCCTCGCCAGCGGGGGCCCGGGCCCTGGCCATGCCTCACTGAGCCAGCCGC
G G S L A S G G P G P G H A S L S Q R L
1270 1290 1310
TGCGCCTCTACCTCGCCGACAGCTGGAACCACTGCGACCTAGTGGCTCTCACCTGCTTCC
R L Y L A D S W N Q C D L V A L T C F L
1330 1350 1370
TCCTGGGCGTGGGCTGCCGCTGACCCCGGTTTGTACCACTGGGCGGCACTGTCTCTCT
L G V G C R L T P G L Y H L G R T V L C
1390 1410 1430
GCATCGACTTCATGGTTTTCACGGTGCGGCTGCTTCACATCTTCACGGTCAACAAACAGC
I D F M V F T V R L L H I F T V N K Q L
1450 1470 1490
TGGGGCCCAAGATCGTCATCGTGAGCAAGATGATGAAGGACGTGTTCTTCTTCTCTCTCT
G P K I V I V S K M M K D V F F F L F F
1510 1530 1550
TOCTCGGCGTGTGGCTGGTAGCCTATGGCGTGGCCACGGAGGGCTCCTGAGGCCACGGG
L G V W L V A Y G V A T E G L L R P R D
1570 1590 1610
ACAGTGACTTCCCAAGTATCTGCGCGCGCTCTTCTACCGTCCCTACCTGCAGATCTTCG
S D F P S I L R R V F Y R P Y L Q I F G
1630 1650 1670
GGCAGATTCCCCAGGAGACATGGACGTGGCCCTCATGGAGCACAGCAACTGCTCGTGGG
Q I P Q E D M D V A L M E H S N C S S E
1690 1710 1730
AGCCCCGGCTTCTGGGCACACCCTCCTGGGGCCAGGCGGGCACCTGCGTCTCCAGTATG

Fig. 9 / continuation 5

P G F W A H P P G A Q A G T C V S Q Y A
1750 1770 1790
CCAACGGCTGGTGGTGGCTGCTCCTCGTCATCTTCCTGCTCGTGGCCAACATCCTGCTGG
N W L V V L L L V I F L L V A N I L L V
1810 1830 1850
TCAACTTGCTCATTGCCATGTTTCAGTTACACATTTCGGCAAAGTACAGGGCAACAGCGATC
N L L I A M F S Y T F G K V Q G N S D L
1870 1890 1910
TCTACTGGAAGGCGCAGCGTTACCGCCTCATCCGGGAATTCCTCTCGGCCCCGCGCTGG
Y W K A Q R Y R L I R E F H S R P A L A
1930 1950 1970
CCCCGCCCTTTATCGTCATCTCCCACTTGCGCCTCCTGCTCAGGCAATTGTGTCAGGCGAC
P P F I V I S H L R L L L R Q L C R R P
1990 2010 2030
CCCGGAGCCCCAGCCGCTCCTCCCGGCCCTCGAGCATTTCCGGGTTTACCTTTCTAAGG
R S P Q P S S P A L E H F R V Y L S K E
2050 2070 2090
AAGCCGAGCGGAAGCTGCTAACGTGGGAATCGGTGCATAAGGAGAACCTTTCTGCTGGCAC
A E R K L L T W E S V H K E N F L L A R
2110 2130 2150
GCGCTAGGGACAAGCGGGAGAGCGACTCCGAGCGTCTGAAGCGCACGTCCCAGAAGGTGG
A R D K R E S D S E R L K R T S Q K V D
2170 2190 2210
ACTTGGCACTGAAACAGCTGGGACACATCCGCGAGTACGAACAGCGCCTGAAAGTCTGG
L A L K Q L G H I R E Y E Q R L K V L E
2230 2250 2270
AGCGGGAGGTCCAGCAGTGTAGCCGCTCCTGGGGTGGGTGGCCGAGGCCCTGAGCCGCT
R E V Q Q C S R V L G W V A E A L S R S
2290 2310 2330
CTGCCCTTGCTGCCCCAGGTGGGCGGCCACCCCTGACCTGCCTGGGTCCAAAGACTGAG
A L L P P G G P P P P D L P G S K D *
2350 2370 2390
CCCTGCTGGCGGACTTCAAGGAGAAGCCCCACAGGGGATTTTGCTCCTAGAGTAAGGCT
2410 2430 2450
CATCTGGGCTCGGCCCCCGCACCTGGTGGCCTTGTCTTGAAGGTGAGCCCCATGTCCAT
2470 2490 2510
CTGGGCCACTGTGAGGACCACCTTTGGGAGTGTCTTACAAACCACAGCATGCCCGG
2530 2550 2570
CTCCTCCAGAACAGTCCCAGCCTGGGAGGATCAAGGCCTGGATCCCGGGCGTTATCC
2590 2610 2630
ATCTGGAGGCTGCAGGGTCTTGGGGTAACAGGGACCACAGACCCCTCACCCTCACAGA
2650 2670 2690
TTCCTCACACTGGGGAATAAAGCCATTTAGAGGAAAAAAAAAAAAAAAAAAAAA

MYLLSDKATSPLSLDAGLGQAPWSDLLLWALLLNRAQMAMYFWEMGSNAVSSALGACLLLRVMARLEPDAEEAARRKDIAFKFEGM
GVDLFGECYRSSEVRAARLLLRRCPWGDATCLQLAMQADARAFFAQDGVQSILLTQKWGDMASTTPIWALVLAFFCPPLIYTRLI
TFRKSEEEPTREELEFDMDSVINGEGPVGTADPAEKTPLGVRQSGRPGCCGGRRCRRWFHFWGVPTIFMGNVVSYLEFL
LLFSRVLLVDFQPAPPGSLELLLYFWAFTLLCEELRQGLSGGGSLASGGPGPGHASLSQRLRLYLADSWNQCDLVALTCFLLGVG
CRLTPGLYHLGRTVLCIDFMVFTVRLHLHIFTVNKQLGPKIVIVSKMMKDVFFLFFLGVWLVAVGVATEGLLRPRDSDFPSILRRV
FYRPYLQIFGQIPQEDMDVALMEHSNCSSEPGFWAHPGAQAGTCVSQYANWLVLVLLVIFLLVANILLVNLIIAMFSYTFGKVQG
NSDLYWKAQRYRLIREFHRSRPAAPPFIVISHRLLLRQLCRRPRSPQSSPALEHFRVYLSKEAERKLLTWESVHKENFLLARAR
DKRESDSERLKRTSQKVDLALKQLGHIREYQRLKVLEREVQQCSRVLGWVAEALSRSALLPPGGPPPPDLPGSKD

A) 10 30 50
ATTAAAGTTTATAAAACAGTGGCTGGATGGTTGGAGGATGCAGGTGGACAGAAGACGTGG
M V G G C R W T E D V E
70 90 110
AGCCTGCAGAAAGTAAAGGAAAAGATGTCTTTCGGGCAGCCAGGCTCAGCATGAGGAACA
P A E V K E K M S F R A A R L S M R N R
130 150 170
GAAGGAATGACACTCTGGACAGCACCCGGACCTGTACTCCAGCGCTCTCGGAGCACAG
R N D T L D S T R T L Y S S A S R S T D
190 210 230
ACTTGTCTTACAGTGAAAGCGCCAGCTTCTACGCTGCCTTCAGGACACAGACGTGCCCAA
L S Y S E S A S F Y A A F R T Q T C P I
250 270 290
TCATGGCTTCTTGGGACTTGGTGAATTTATTCAGCAAATTTTAAAGAAACGAGAATGTG
M A S W D L V N F I Q A N F K K R E C V
310 330 350
TCTTCTTTACCAAAGATCCAAGGCCACGGAGAATGTGTGCAAGTGTGGCTATGCCCAGA
F F T K D S K A T E N V C K C G Y A Q S
370 390 410
GCCAGCACATGGAAGGCCACCCAGATCAACCAAGTGAGAAATGGAACACAGAAACACA
Q H M E G T Q I N Q S E K W N Y K K H T
430 450 470
CCAAGGAATTTCTACCGACGCTTTGGGGATATTGAGTTTGAGACACTGGGGAAGAAAG
K E F P T D A F G D I Q F E T L G K K G
490 510 530
GGAAGTATATACGTCTGTCTGCGACACGGACGCGGAAATCCTTTACGAGCTGCTGACCC
K Y I R L S C D T D A E I L Y E L L T Q
550 570 590
AGCACTGGCACCTGAAAACACCCAACCTGGTCATTTCTGTGACCGGGGGCGCAAGAACT
H W H L K T P N L V I S V T G G A K N F
610 630 650
TCGCCCTGAAGCGCGCATGCGCAAGATCTTCAGCCGCTCATCTACATCGCGCAGTCCA
A L K F R M R K I F S R L I Y I A Q S K
670 690 710
AAGGTGCTTGGATTCTCACGGGAGGCACCCATTATGGCCTGATGAAGTACATCGGGGAGG
G A W I L T G G T H Y G L M K Y I G E V
730 750 770
TGGTGAGAGATAACACCATCAGCAGGAGTTTCAGAGGAGAATATTGTGGCCATTGGCATAG
V R D N T I S R S S E E N I V A I G I A
790 810 830
CAGCTTGGGGCATGGTCTCCAACCGGACACCTCATCAGGAATTGCGATGCTGAGGGCT
A W G M V S N R D T L I R N C D A E G Y
850 870 890
ATTTTTAGCCAGTACCTTATGGATGACTTCACAAGAGATCCACTGTATATCCTGGACA
F L A Q Y L M D D F T R D P L Y I L D N
910 930 950
ACAACCACACACATTTGCTGCTCGTGGACAATGGCTGTCATGGACATCCCACTGTGGAAG
N H T H L L L V D N G C H G H P T V E A
970 990 1010
CAAAGCTCCGGAATCAGCTAGAGAAGTATATCTCTGAGCGCACTATTCAAGATTCCAAC
K L R N Q L E K Y I S E R T I Q D S N Y
1030 1050 1070
ATGGTGGCAAGATCCCCATTGTGTGTTTGGCCAAGGAGGTGAAAGAGACTTTGAAG
G G K I P I V C F A Q G G G K E T L K A
1090 1110 1130
CCATCAATACCTCCATCAAAAATAAAATTCCTTGTGTGGTGGTGAAGGCTCGGGCCAGA
I N T S I K N K I P C V V V E G S G Q I
1150 1170 1190
TCGCTGATGTGATCGCTAGCCTGGTGGAGGTGGAGGATGCCCTGACATCTTCTGCCGTCA
A D V I A S L V E V E D A L T S S A V K
1210 1230 1250

Fig. 10 / continuation 1

AGGAGAAGCTGGTGCCTTTTACCCGACGGTGTCCCGGCTGCCTGAGGAGGAGACTG
E K L V R F L P R T V S R L P E E E T E
1270 1290 1310
AGAGTTGGATCAATGGCTCAAAGAAATTCTCGAATGTTCTCACCTATTAACAGTTATTA
S W I K W L K E I L E C S H L L T V I K
1330 1350 1370
AAATGGAAGAGCTGGGGATGAAATTGTGAGCAATGCCATCTCTACGCTCTATACAAG
M E E A G D E I V S N A I S Y A L Y K A
1390 1410 1430
CCTTCAGCACCAGTGAGCAAGACAAGGATAACTGGAATGGGCAGCTGAAGCTTCTGCTGG
F S T S E Q D K D N W N G Q L K L L L E
1450 1470 1490
AGTGGAAACCAGCTGGACTTAGCCAATGATGAGATTTTCACCAATGACCGCCGATGGGAGA
W N Q L D L A N D E I F T N D R R W E K
1510 1530 1550
AGAGCAAACCGAGGCTCAGAGACACAATAATCCAGGTCACATGGCTGGAAATGGTAGAA
S K P R L R D T I I Q V T W L E N G R I
1570 1590 1610
TCAAGGTTGAGAGCAAAGATGTGACTGACGGCAAAGCCTCTTCTCATATGCTGGTGGTTC
K V E S K D V T D G K A S S H M L V V L
1630 1650 1670
TCAAGTCTGCTGACCTTCAAGAAGTCATGTTTACGGCTCTCATAAAGGACAGACCCAAGT
K S A D L Q E V M F T A L I K D R P K F
1690 1710 1730
TTGTCCGCTCTTTCTGAGCAATGGCTTGAACCTACGGAAGTTTCTCACCCATGATGTCC
V R L F L E N G L N L R K F L T H D V L
1750 1770 1790
TCACTGAACCTCTTCTCAACCACTTCAGCAAGCTTGTGTACCGGAATCTGCAGATCGCCA
T E L F S N H F S T L V Y R N L Q I A K
1810 1830 1850
AGAATTCCTATAATGATGCCCTCCTCAGCTTTGTCTGGAAACTGGTTGCGAACTTCCGAA
N S Y N D A L L T F V W K L V A N F R R
1870 1890 1910
GAGGCTTCCGGAAGGAAGACAGAAATGGCCGGGACGAGATGGACATAGAACTCCACGACG
G F R K E D R N G R D E M D I E L H D V
1930 1950 1970
TGTCTCTATTACTCGGCACCCCTGCAAGCTCTCTTCATCTGGGCCATTCTTCAGAATA
S P I T R H P L Q A L F I W A I L Q N K
1990 2010 2030
AGAAGGAACCTCTCCAAAGTCATTTGGGAGCAGACCAGGGGCTGCACTCTGGCAGCCCTGG
K E L S K V I W E Q T R G C T L A A L G
2050 2070 2090
GAGCCAGCAAGCTTCTGAAGACTCTGGCCAAAGTGAAGAACGACATCAATGCTGCTGGGG
A S K L L K T L A K V K N D I N A A G E
2110 2130 2150
AGTCCGAGGAGCTGGCTAATGAGTACGAGACCCGGGCTGTTGGTGAGTCCACAGTGTGGA
S E E L A N E Y E T R A V G E S T V W N
2170 2190 2210
ATGCTGTGGTGGGCGCGGATCTGCCATGTGGCAGACATTGCCAGCGGCACTCATAGAC
A V V G A D L P C G T D I A S G T H R P
2230 2250 2270
CAGATGGTGGAGAGCTGTTCACTGAGTGTACAGCAGCGATGAAGACTTGGCAGAACAGC
D G G E L F T E C Y S S D E D L A E Q L
2290 2310 2330
TGCTGGTCTATTCTGTGAAGCTTGGGGTGAAGCAACTGTCTGGAGCTGGCGGTGGAGG
L V Y S C E A W G G S N C L E L A V E A
2350 2370 2390
CCACAGACCAGCATTTCATCGCCAGCCTGGGGTCCAGAATTTCTTCTAAGCAATGGT
T D Q H F I A Q P G V Q N F L S K Q W Y
2410 2430 2450
ATGGAGAGATTTCCCGAGACACCAAGAACTGGAAGATTATCCTGTGTCTGTTATTATAC
G E I S R D T K N W K I I L C L F I I P

Fig. 10 / continuation 2

2470 2490 2510
CCTTGGTGGGCTGTGGCTTTGTATCATTAGGAAGAAACCTGTCGACCAAGCACAAGAAGC
L V G C G F V S F R K K P V D K H K K L
2530 2550 2570
TGCTTTGGTACTATGTGGCGTTCTTCACCTCCCCCTCGTGGTCTTCTCCTGGAATGTGG
L W Y Y V A F F T S P F V V F S W N V V
2590 2610 2630
TCTTCTACATCGCCTTCCTCCTGCTGTTTGCCTACGTGCTGCTCATGGATTCCATTGGG
F Y I A F L L L F A Y V L L M D F H S V
2650 2670 2690
TGCCACACCCCCGAGCTGGTCTGCTGCTGCTGCTTTGTCTCTTCTGTGATGAAG
P H P P E L V L Y S L V F V L F C D E V
2710 2730 2750
TGAGACAGGGCCGGCCGGCTGCTCCAGTGCGGGGCGGCCAAGCCACGCCACCCCGGA
R Q G R P A A P S A G P A K P T P T R N
2770 2790 2810
ACTCCATCTGGCCCCGCAAGCTCCACAGCAGCCCCGGTCCCGCTCAGCCACTCCTTCC
S I W P A S S T R S P G S R S R H S F H
2830 2850 2870
ACACTTCCCTGCAAGCTGAGGGTGCCAGCTCTGGCCTTGGCCAGCCAGAAAGGGGTGGA
T S L Q A E G A S S G L G Q P R K G W T
2890 2910 2930
CATTAAAAATCTGGAATGGTTGATATTTCCAAGCTGCTGATGCTCCCTCTCTGTCCCTT
F K N L E M V D I S K L L M S L S V P F
2950 2970 2990
TCGTACGCACTGGTACGTAATGGGTGAATTATTTACTGACCTGTGGAATGTGATGG
C T Q W Y V N G V N Y F T D L W N V M D
3010 3030 3050
ACACGCTGGGGCTTTTTTACTTCATAGCAGGAATTGTATTTGGCAAGGGATCCTTAGGC
T L G L F Y F I A G I V F R Q G I L R Q
3070 3090 3110
AGAATGAGCAGCGCTGGAGGTGGATATTCGGTTCGGTCTACGAGCCCTACCTGGCCA
N E Q R W R W I F R S V I Y E P Y L A M
3130 3150 3170
TGTTGGCCAGGTGCCAGTGACGTGGATGGTACCACGTATGACITTGCCCACTGCACCT
F G Q V P S D V D G T T Y D F A H C T F
3190 3210 3230
TCACATGGGAATGAGTCCAAGCCACTGTGTGTGGAGCTGGATGAGCACAACCTGCCCGGT
T G N E S K P L C V E L D E H N L P R F
3250 3270 3290
TCCCGAGTGGATCAACATCCCGCTGGTGTGCATCTACATGTTATCCACCAACATCCTGC
P E W I T I P L V C I Y M L S T N I L L
3310 3330 3350
TGGTCAACCTGCTGGTGCCTATGTTGGCTACACGGTGGGCACCGTCCAGGAGAACAATG
V N L L V A M F G Y T V G T V Q E N N D
3370 3390 3410
ACCAGGTCTGGAAGTTCAGAGGTACTTCTGGTGCAGGAGTACTGCAGCCGCTCAATA
Q V W K F Q R Y F L V Q E Y C S R L N I
3430 3450 3470
TCCCTTCCCTTCATCGTCTTCTGCTTACTTCTACATGGTGGTGAAGAAGTGTCTCAAGT
P F P F I V F A Y F Y M V V K K C F K C
3490 3510 3530
GTTGCTGCAAGGAGAAAAACATGGAGTCTTCTGTCTGCTGTGAGTGGTTATCCATGTGT
C C K E K N M E S S V C C E W F I H V Y
3550 3570 3590
ACTTGGGATCAGAAGCAGCGATTAAATTCAGGGAAGGATGCCTGCATCCAGTGAATGGAA
L G S E A A I N F R E G C L H P V I G S
3610 3630 3650
GCTGGACCCAGGCTGGTGGTCTGGACATCCACACGCAATCTCACATGCAGTGCCGGCT
W T P G W L V W T S T R I L T C S A G W
3670 3690 3710
GGCCAGCAGCAGGGAGTCTCAGTGTCAACACACATAGCAGCTGGGTTCCTGCAAAAAGCA

Fig. 10 / continue on 3

P A A G S L S V T T H S S W V P A K S S
 3730 3750 3770
 GCAAGTCACAGGCCACCCAGACAGAACGGGTAGAGAATGTGACTCTGCTTCTGGGTGGG
 K S Q A H P D R T G R E C D S A S G W E
 3790 3810 3830
 AAGGACAGCCTGCCCCGGTGGGTGGAAGAATCCGTGGCCCTGTTTGGCCATCGTGGCCCTG
 G Q P A R W V E E S V A L F G H R G P V
 3850 3870 3890
 TTTGGCCACCTACCACTCTAGGCATCACTGAGCTGAATGCGCCGGTCTCTGA
 W P P T T L G I T E L N A P V L *

MVGGCRWTEDEPAEVKEKMSFRAARLSMRNRNDTLDSTRITLYSSASRSTDLSYSESASFYAAFRQTQCPIMASWDLVNFQANF
 KKRECVFTTKDSKATENVCKQGYAQSOHMEGTQINQSEKWNKYKHTKEFPTDAFGDIQFETLGKKGKYIRLSCDTDAEILYELLTQ
 HWHLKTPNLVISVTGGAKNFALKPRMRKIFSRLLIYIAQSKGAWILTTGGTHYGLMKYIGEVVRDNTISRSSEENIVAIGIAWGMVS
 NRDTLIRNCDAEGYFLAQYLMDDFTRDPLYILDNNHTHLLVDNGCHGHPTVEAKLRNQLKEYISERTIQDSNYGGKIPIVCFAQG
 GKETLKAINTSIKNKIPCVVVEGSGQIADVIASLVEVEDALTSSAVKEKLVRFLPRTVSRLPEEETESWIKWLKEILECSHLLTV
 IKMEEAGDEIVSNAISYALYKAFSTSEQDKDNWNGQLKLLLEWNQLDLANDEIFTNDRRWEKSKPLRDTIIQVTWLENGRIKVES
 KDVTGKASSHMLVVLKSADLQEVMTALIKDRPKFVRLFLENGLNLRKFLTHDVLTELFNSHFSTLVYRNQLIAKNSYNDALLTF
 VWKLVANFRRGFRKEDRNGRDEMDELHDVSPITRHPLOALFIWAILQNKKELSKVIWEQTRGCTLAALGASKLLKTLAKVKNDIN
 AAGESEELANEYETRAVGESTVWNAVVGADLPCGTDIASGTHRPDGGELFTECYSSDEDLAEQLLVYSCEAWGGSNCLELAVEATD
 QHFIAQPGVQNFELSKQWYGEISRDTKNWKIILCLFIIPLVGCGFVSFRKKPVDKHKLLWYVAFFTSPFVVFVSWNVVFYIAFLLL
 FAYVLLMDFHSVPHPELVLYSLVFLFCDEVQRPAAPSAGPAKPTPTRNSIWPASSTRSPGSRSRHSFHTSLQAEGASSGLGQ
 PRKGWTFKNLEMVDISKLLMSLSVPFCTQWYVNGVNYFTDLWNVMDTLGLFYFIAGIVFRQGI LRONEQRWRWIFRSVIYEPYLA
 FGQVPSDVEDGTYDFAHCTFTGNESKPLCVLDEHNLPRFPEWITIPLVCIYMLSTNILLVNLVAMFGYTVGTVQENNDQVWKFO
 RYFLVQEYCSRLNIPFPFIVFAYFYMVVKCFKCCCKEKNMESSVCCWEFIHVYLGSEAINFREGCLHPVIGSWTPGWLVTSTR
 ILTCSAGWPAAGSLSVTTHSSWVPAKSSKSAHPDRTGRECDSSAGWEGQPARWVEESVALFGHRGPVWPPTTLGITELNAPVL

B.

2290 2310 2330 Q L
 TGCTGGTCTATTCTGTGAAGCTTGGGGTGGGAAGCAACTGTCTGGAGCTGGCGGTGGAGG
 L V Y S C E A W G G S N C L E L A V E A
 2350 2370 2390
 CCACAGACCAGCATTTTCATCGCCAGCCTGGGGTCCAGAATTTTCTTTCTAAGCAATGGT
 T D Q H F I A Q P G V Q N F L S K Q W Y
 2410 2430 2450
 ATGGAGAGATTTCCCGAGACACCAAGAACTGGAAGATTATCCTGTGTCTGTTTATTATAC
 G E I S R D T K N W K I I L C L F I I P
 2470 2490 2510
 CCTTGGTGGGCTGTGGCTTTGTATCATTTAGGAAGAAACCTGTGCGACAAGCACAAGAAGC
 L V G C G F V S F R K K P V D K

Figure 11:

a.) Trp10b cDNA and derived amino acid sequence

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      10      30      50
ATGAAATCCTTCCTTCCTGTCCACACCATCGTGCTTATCAGGGAGAATGTGTGCAAGTGT
M K S F L P V H T I V L I R E N V C K C
      70      90      110
GGCTATGCCCAGAGCCAGCACATGGAAGGCACCCAGATCAACCAAAGTGAGAAATGGAAC
G Y A Q S Q H M E G T Q I N Q S E K W N
      130      150      170
TACAAGAAACACACCAAGGAATTTCTACCGACGCCTTTGGGGATATTTCAGTTTGAGACA
Y K K H T K E F P T D A F G D I Q F E T
      190      210      230
CTGGGGAAGAAAGGAAGTATATACGTCTGTCTCGACACGACGCGGAAATCCTTTAC
L G K K G K Y I R L S C D T D A E I L Y
      250      270      290
GAGCTGCTGACCCAGCACTGGCACCTGAAAACACCCAACCTGGTCATTTCTGTGACCGGG
E L L T Q H W H L K T P N L V I S V T G
      310      330      350
GGCGCCAAGAACTTCGCCCTGAAGCCGCGCATGCGCAAGATCTTCAGCCGGCTCATCTAC
G A K N F A L K P R M R K I F S R L I Y
      370      390      410
ATCGCGCAGTCCAAAGGTGCTTGGATTCTCACGGGAGGCACCCATTATGGCCTGATGAAG
I A Q S K G A W I L T G G T H Y G L M K
      430      450      470
TACATCGGGGAGGTGGTGAGAGATAACACCATCAGCAGGAGTTTCAGAGGAGAATATTGTG
Y I G E V V R D N T I S R S S E E N I V
      490      510      530
GCCATTGGCATAGCAGCTTGGGGCATGGTCTCCAACCGGGACACCCTCATCAGGAATTGC
A I G I A A W G M V S N R D T L I R N C
      550      570      590
GATGCTGAGGGCTATTTTTTAGCCCACTACCTTATGGATGACTTCACAAGAGATCCACTG
D A E G Y F L A Q Y L M D D F T R D P L
      610      630      650
TATATCCTGGACAACAACCACACACATTTGCTGCTCGTGGACAATGGCTGTTCATGGACAT
Y I L D N N H T H L L L V D N G C H G H
      670      690      710
CCCACTGTGCAAGCAAAGCTCCGGAATCAGCTAGAGAAGTATATCTCTGAGCGCACTATT
P T V E A K L R N Q L E K Y I S E R T I
      730      750      770
CAAGATTCCAATATGGTGGCAAGATCCCCATTGTGTGTTTTGCCCAAGGAGGTGGAAAA
Q D S N Y G G K I P I V C F A Q G G G K
      790      810      830
GAGACTTTGAAAGCCATCAATACCTCCATCAAAAATAAAATTCCTTGTGTGGTGGTGGAA
E T L K A I N T S I K N K I P C V V V E
      850      870      890
GGCTCGGGCCAGATCGCTGATGTGATCGCTAGCCTGGTGGAGGTGGAGGATGCCCTGACA
G S G Q I A D V I A S L V E V E D A L T
      910      930      950
TCTTCTGCCGTCAAGGAGAAGCTGGTGCCTTTTTACCCCGCACGGTGTCCCGGCTGCCT
S S A V K E K L V R F L P R T V S R L P
      970      990      1010
GAGGAGGAGACTGAGAGTTGGATCAAATGGCTCAAAGAAATTCTCGAATGTTCTCACCTA
E E E T E S W I K W L K E I L E C S H L
      1030      1050      1070
TTAACAGTTATTAAATGGAAGAAGCTGGGGATGAAATTGTGAGCAATGCCATCTCCTAC
L T V I K M E E A G D E I V S N A I S Y
      1090      1110      1130
GCTCTATACAAAGCCTTCAGCACCAAGTGAAGACAAGGATAACTGGAATGGGCAGCTG
A L Y K A F S T S E Q D K D N W N G Q L

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Fig. 11 (Continuation)

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2410      2430      2450
AGAAACTTAGGACCCAAGATTATAATGCTGCAGAGGATGCTGATCGATGTGTTCTTCTTC
R N L G P K I I M L Q R M L I D V F F F
2470      2490      2510
CTGTTCCCTCTTTGCGGTGTGGATGGTGGCCTTTGGCGTGGCCAGGCAAGGGATCCTTAGG
L F L F A V W M V A F G V A R Q G I L R
2530      2550      2570
CAGAATGAGCAGCGCTGGAGGTGGATATTCCGTTTCGGTCATCTACGAGCCCTACCTGGCC
Q N E Q R W R W I F R S V I Y E P Y L A
2590      2610      2630
ATGTTCCGGCCAGGTGCCCAGTGACGTGGATGGTACCACGTATGACTTTGCCCCTGCACC
M F G Q V P S D V D G T T Y D F A H C T
2650      2670      2690
TTCCTGCGGAATGAGTCCAAGCCACTGTGTGTGGAGCTGGATGAGCACAACCTGCCCGG
F T G N E S K P L C V E L D E H N L P R
2710      2730      2750
TTCCCCGAGTGGATCACCATCCCCCTGGTGTGCATCTACATGTTATCCACCAACATCCTG
F P E W I T I P L V C I Y M L S T N I L
2770      2790      2810
CTGGTCAACCTGCTGGTCGCCATGTTTGGCTACACGGTGGGCACCGTCCAGGAGAACAAAT
L V N L L V A M F G Y T V G T V Q E N N
2830      2850      2870
GACCAGGTCTGGAAGTTCAGAGGTACTTCTGGTGCAGGAGTACTGCAGCCGCCTCAAT
D Q V W K F Q R Y F L V Q E Y C S R L N
2890      2910      2930
ATCCCCCTTCCCCTTCATCGTCTTTCGCTTACTTCTACATGGTGGTGAAGAAGTGCTTCAAG
I P F P F I V F A Y F Y M V V K K C F K
2950      2970      2990
TGTTGCTGCAAGGAGAAAAACATGGAGTCTTCTGTCTGCTGTTTCAAAAATGAAGACAAT
C C C K E K N M E S S V C C F K N E D N
3010      3030      3050
GAGACTCTGGCATGGGAGGGTGTGATGAAGGAAACTACCTTGTCAAGATCAACACAAAA
E T L A W E G V M K E N Y L V K I N T K
3070      3090      3110
GCCAACGACACCTCAGAGGAAATGAGGCATCGATTAGACAACTGGATACAAAGCTTAAT
A N D T S E E M R H R F R Q L D T K L N
3130      3150
GATCTCAAGGGTCTACTGAAAGAGATTGCTAATAAAATCAAATAG
D L K G L L K E I A N K I K *

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b.) Trp10 protein:

MKSFLPVHTIVLIRENVCKCGYAQSQHMEGTQINQSEKWNKKHTKEFPTDAFGDIQFETLGKKGKYIRLSCDTDAEILY
ELLTQHWHLKTPNLVISVTGGAKNFALKPRMRKIFSRLIYIAQSKGAWILTGGTHYGLMKYIGEVVRDNTISRSEENIV
AIGIAAWGMVSNRDTLIRNCDAEGYFLAQYLMDDFTDRPLYILDNNHTHLLLVNDNGCHGHPTVEAKLRNQLEKYISERTI
QDSNYGGKIPVCFQAQGGGKETLKAINTSIKNKIPCVVVEGSGQIADVIALSVEVEDALTSSAVKEKLVRFLPRTVSRLP
EEETESWIKWLKEILECSHLLTVIKMEEAGDEIVSNAISYALYKAFSTSEQDKDNWNGQLKLLLEWNQLDLANDEIFTND
RRWESADLQEVMTALIKDRPKFVRLFLENLNLRKFLTHDVLTELFSTLHVYRNQLIAKNSYNDALLTFVWKLVAN
FRGFRKEDRNRDEMDIELHDVSPITRHLPLQALFIWAILQNKELSKVIWEQTRGCTLAALGASKLLKTLAKVKNDINA
AGESEELANEYETRAVELFTECYSSDEDLAEQLLVYSCEAWGGSNCLELAVEATDQHFIAQPGVQNFSLKQWYGEISRDT
KNWKIILCLFIIPLVGC GFVSFRKKPVDKHKLLWYYVAFFTSPFVVSFVSWNVVFYIAFLLLFAYVLLMDFHSPHPPELV
LYSLVFLFCDEVQRQWYVNGVNYFTDLWNVMDTLGLFYFIAGIVFRLHSSNKSSLYSGRVIFCLDYIIIFTLRLIHIFTVS
RNLGPKIIMLQRMILIDVFFFLFLFAVWMVAFGVARQGI LRQNEQRWRWIFRSVIYEPYLA MFGQVPSDVGTTDYDFAHCT
FTGNESKPLCVELDEHNLPRFPEWITIPLVCIYMLSTNILLVNLVAMFGYTVGTQENNDQVWKFQRYFLVQYEC SRLN
IPFPFIVFAYFYMVVKCFKCCCKEKNMESSVCCFKNEDNETLAWEGVMKENYLVKINTKANDTSEMRHRFRQLD TKLN
DLKGLLKEIANKIK

Figs. 12A and 12B

The Trp8 gene is expressed in endometrial or uterine cancer, but not in normal endometrium

Endometrial cancer:

A



B



Figs. 12C and 12D



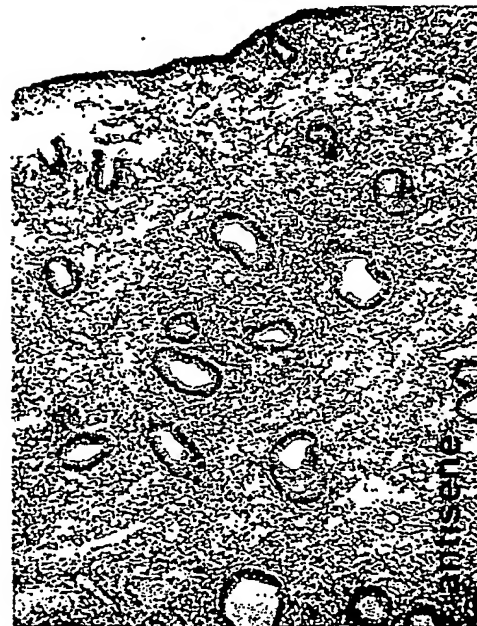
Figs. 12E and 12F

Endometrium:

F



E



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Fig. 13

Expression of human Trp 9 and Trp 10

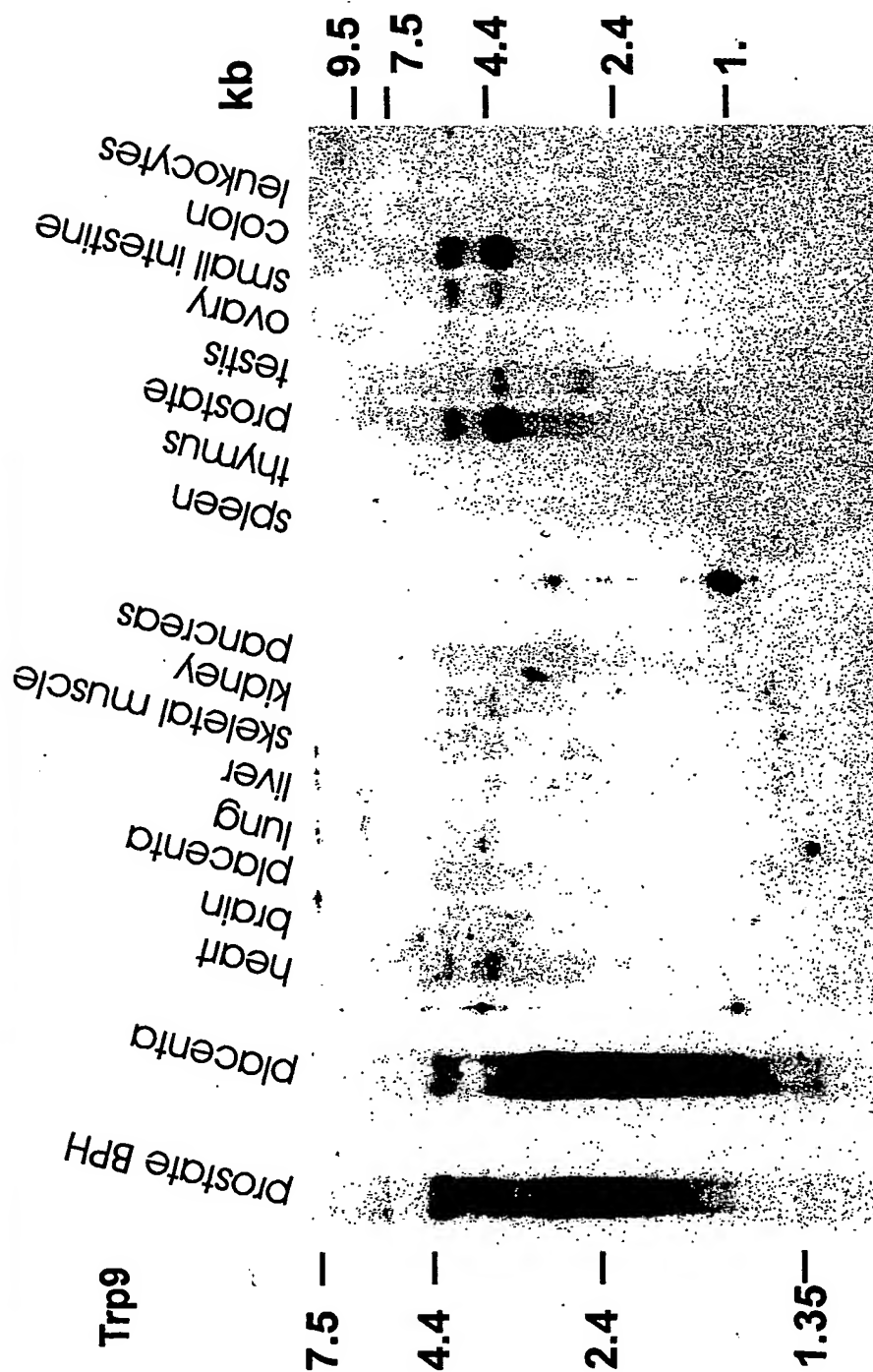
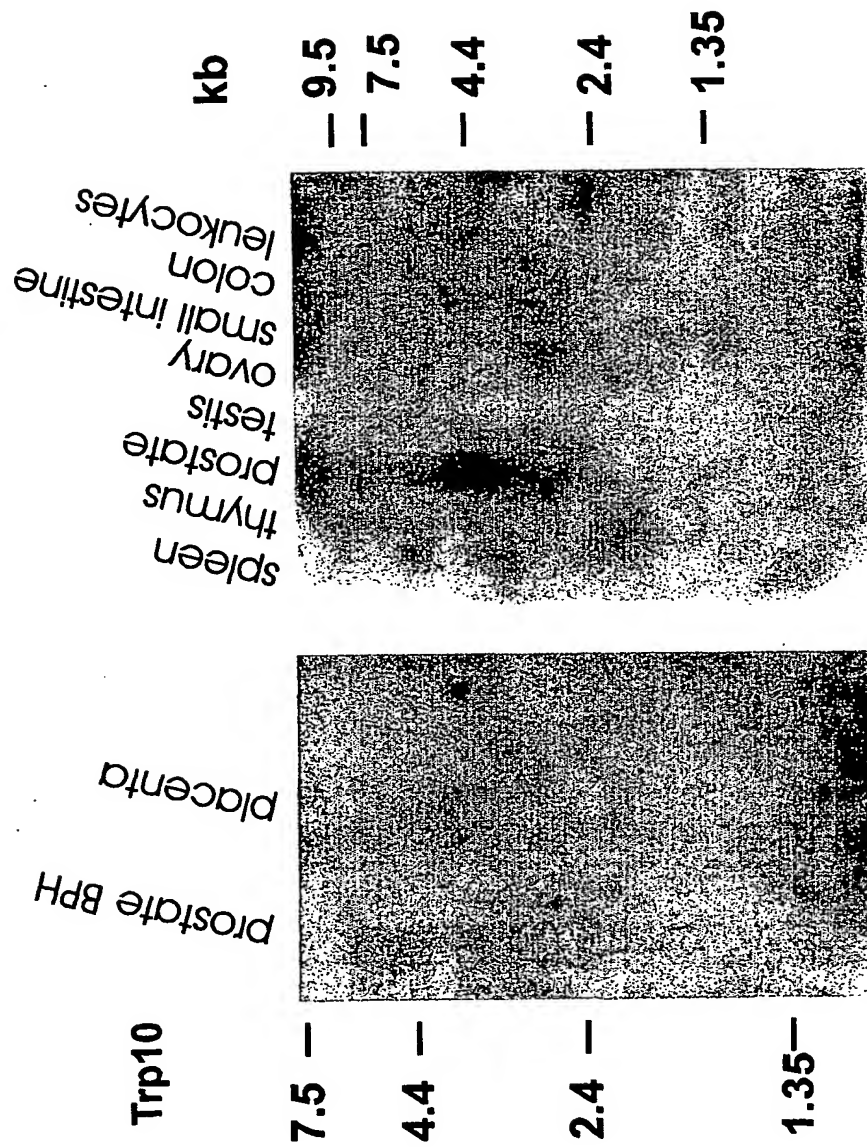
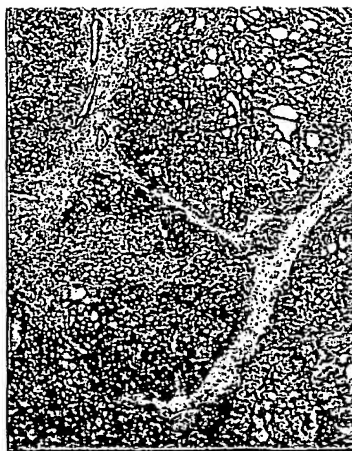


Fig. 13 / Continuation 1

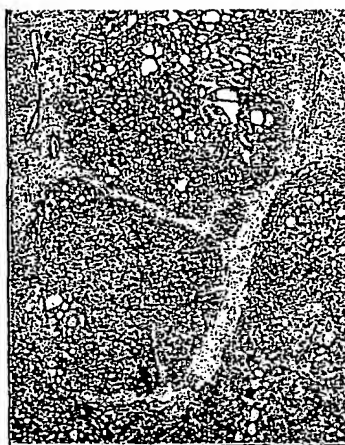


Figs. 14A, 14B, 14F and 14G

Expression of Trp10 transcripts and Trp10-antisense transcripts
in human prostate cancer and in malignant melanoma



A



F



B



G

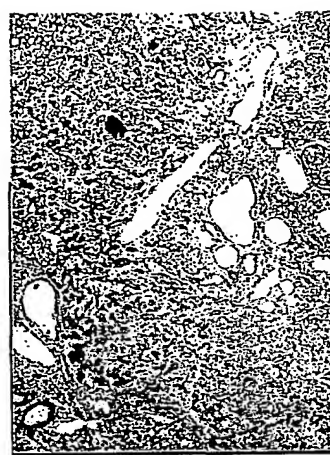
H



I



J



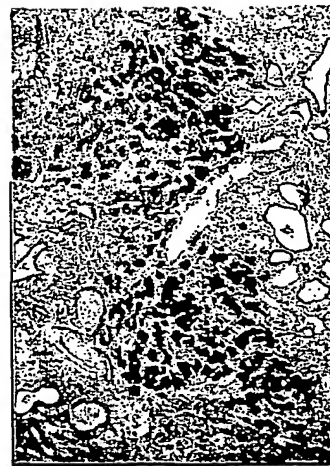
C



D



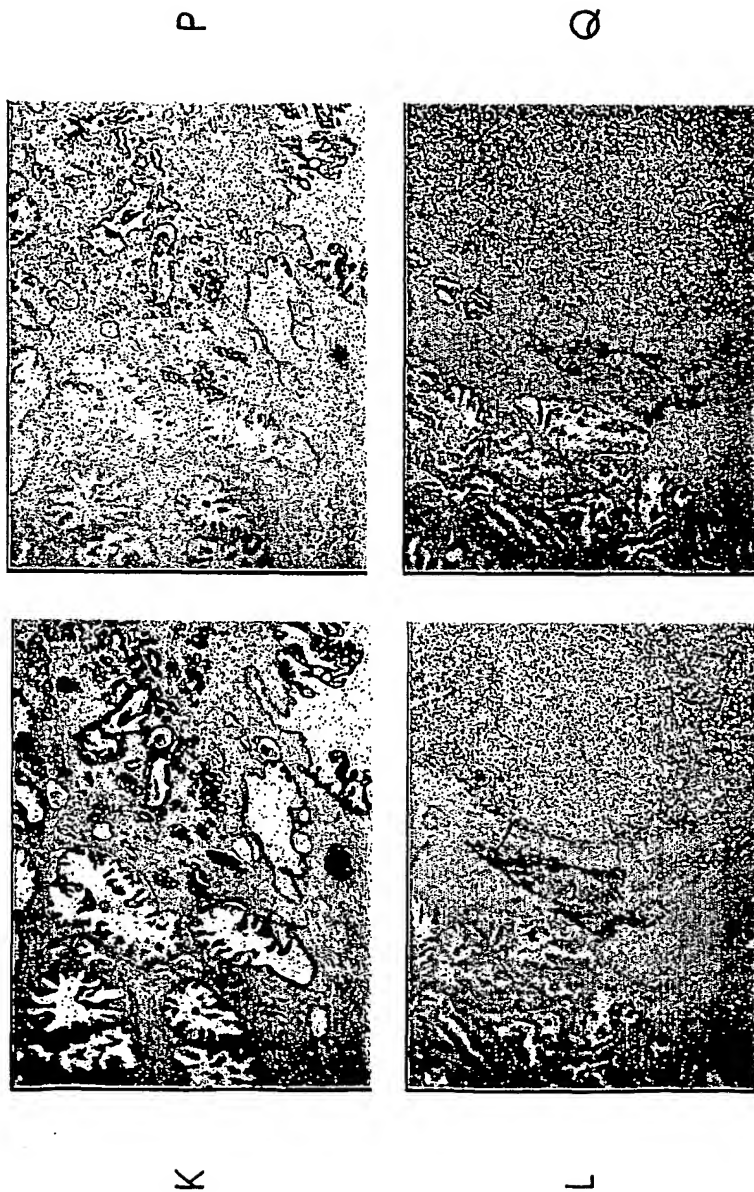
E

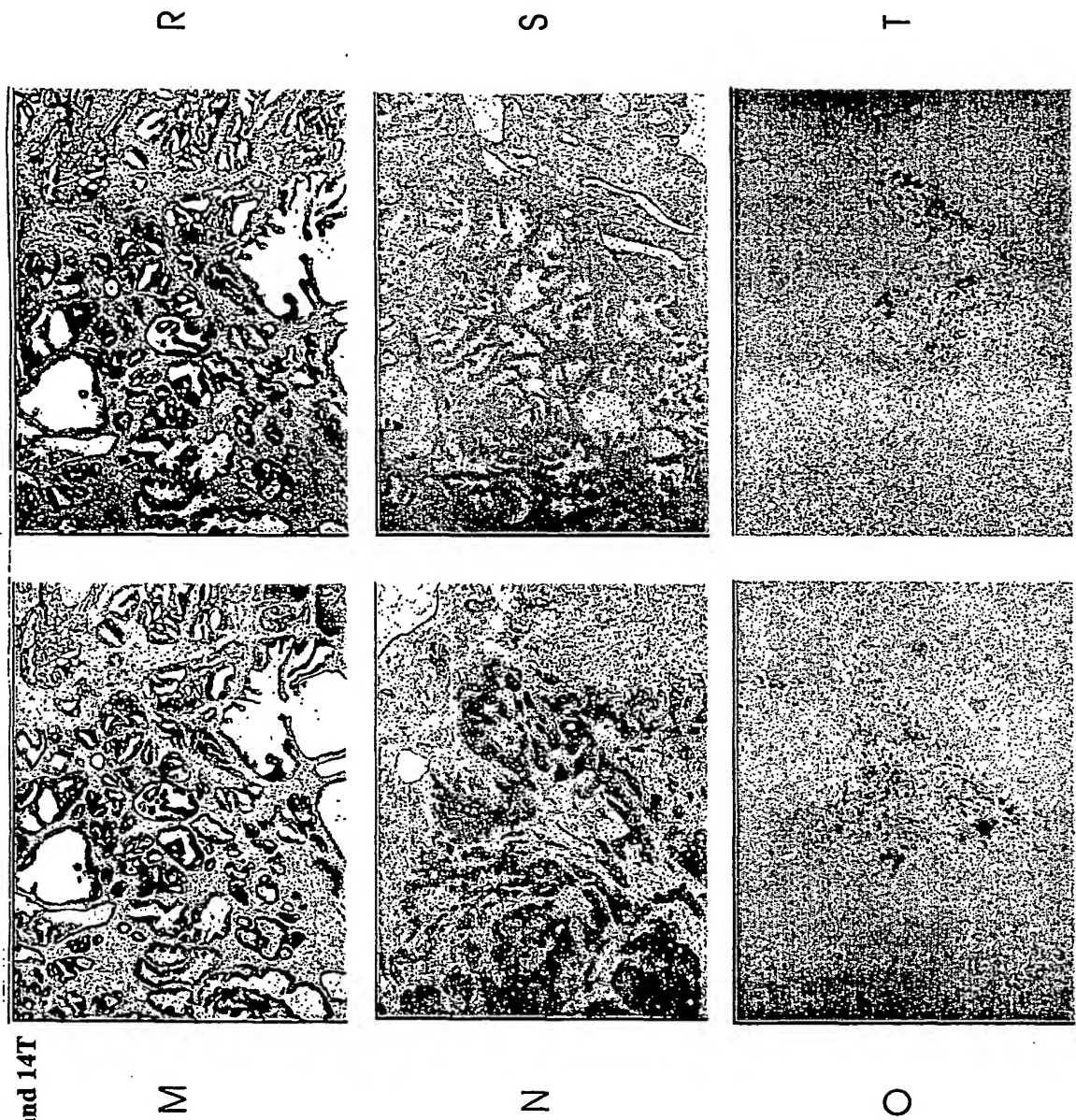


Figs. 14C, 14D, 14E, 14H, 14I and 14J

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Figs. 14K, 14L, 14P and 14Q





Figs. 14M, 14N, 14O, 14R, 14S and 14T

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